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A BOTANICAL AND CHEMICAL STUDY OF BIKUKULLA EXIMIA, WITH A KEY TO NORTH AMERICAN SPECIES OF BIKUKULLA [1]

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INTRODUCTION

In 1921 a study was made of the alkaloids of *Bikukulla cucullaria* and *B. canadensis* in relation to their poisonous properties to grazing animals [2] and from the former there was isolated a new and extremely toxic alkaloid to which was given the name cucullarine. *B. canadensis* was also found to be poisonous but to a less degree. In view of these results it was thought worth while to make a chemical study of *B. eximia*, since no such study had previously been reported.

All the members of the Bikukulla group on which chemical work has been reported are known to contain alkaloids, which is not surprising since they are closely allied to the poppy family. Few of the alkaloids, however, have been examined with sufficient care to prevent confusion as to their identity, and, with the exception of protopine, dicentrine, cucullarine, and an unnamed alkaloid isolated from *B. canadensis*, practically nothing is known about their toxicity.

BIKUKULLA EXIMIA

BOTANICAL DESCRIPTION

Bikukulla eximia (Ker) Millsp. Fringed bleedingheart. Also known as wild bleedingheart, staggerweed, and turkey corn. (Fig. 1.) Perennial, smooth herb, with dark-green basal leaves ternately compound, finely cut into oblong or ovate segments; scaly rootstocks; flower pedicels rising from the root 1 to 2 feet high, taller than the leaves, in compound lengthened clusters; flowers flattened, usually withering and persistent; sepals 2, scalelike; petals 4, the outer produced into rounded sacs at the base, 2 inner petals crested; stamens 6, in two groups, their filaments often united; pods several seeded, splitting; seeds black, warty, crested. Habitat, mountain rocks and river gorges.

This species was found at Junius, Seneca County, N. Y., by H. P. Startwell more than a century ago, but it has never been found so far north since. Otherwise the plant ranges in the southern Appalachian Mountains from Wills Mountain, Allegany County, Md., south to Georgia.

EXPERIMENTAL WORK

The material on which the work was done consisted of leaves and twigs of *Bikukulla eximia* collected near Deerfield in Augusta County, Va., by the senior writer in June, 1923. This material, which was in

[1] Received for publication Mar. 19, 1929; issued October, 1929.

[2] BLACK, O. F., EGGLESTON, W. W., KELLY, J. W., and TURNER, H. C. POISONOUS PROPERTIES OF BIKUKULLA CUCULLARIA (DUTCHMAN'S-BREECHES) AND B. CANADENSIS (SQUIRREL-CORN). Jour. Agr. Research 23: 69-78, illus. 1923.

good condition when received, was ground to a moderately fine powder. A few grams of the powdered product was extracted with Prollius's solution, and the evaporated extract was taken up with

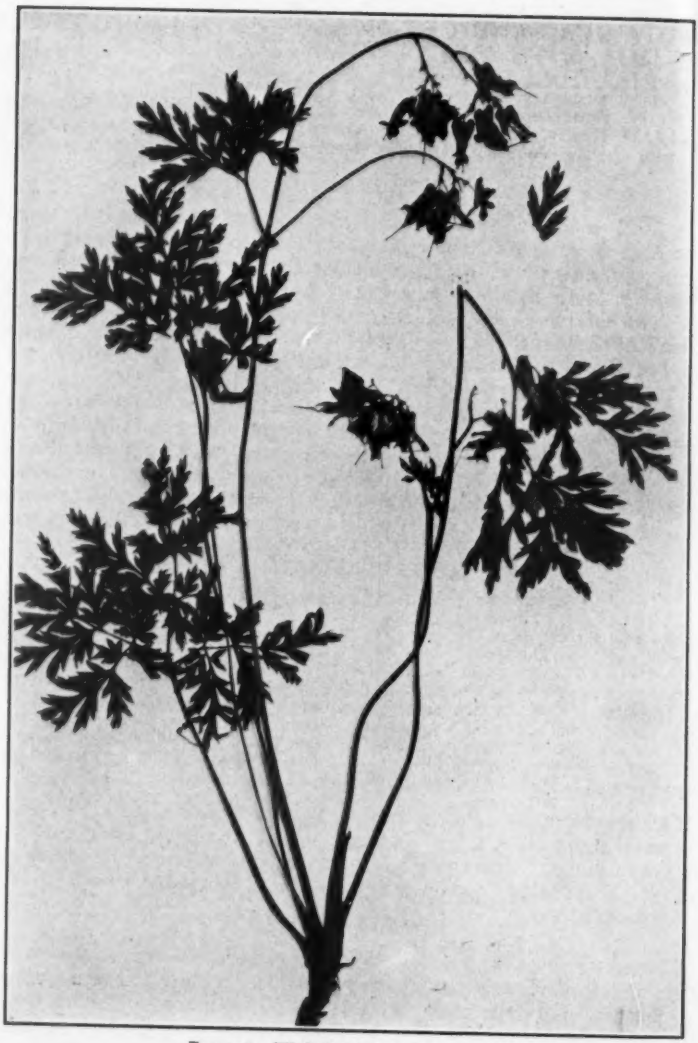

FIGURE 1.—*Bikukulla eximia* in flower. × ⅓

dilute hydrochloric acid, filtered, and tested with Mayer's reagent. This gave a heavy flocculent precipitate, indicating the presence of alkaloids in quantity.

As the primary object of the work was to find out whether this plant is toxic to animals, the next step was to make a preparation that would contain the total alkaloids from a known weight of the plant and to test the toxicity of the preparation by administering definite doses of it to white mice. To this end 30 gm. of the finely ground plant was moistened with alcohol and acetic acid, packed in a percolator, and thoroughly extracted with 95 per cent alcohol. The resulting extract was evaporated by distillation under reduced pressure, and the residue was taken up with dilute acetic acid and then made alkaline with ammonia. The alkaloid was shaken out with chloroform, which entirely removed it. The chloroform extract was concentrated to a few cubic centimeters, and then extracted with dilute acetic acid. The last extract was made alkaline with ammonia and again shaken out with chloroform, the solvent was evaporated in a tared dish, and the residue was weighed. The residue consisted of an amorphous light-yellow substance weighing 0.7 gm., equivalent to 2.3 per cent of the dry plant. The crude alkaloid was next treated with very dilute hydrochloric acid, which, it was observed, did not effect complete solution. The insoluble portion was dried and weighed. One-half cubic centimeter of the solution, representing 8 mgm. of alkaloid, was then injected subcutaneously into a mouse weighing about 15 gm. No marked symptoms of any kind were observed in the animal, with the possible exception of a slight drowsiness. As 8 mgm. is a very large dose for an animal of 15 gm. weight, it was concluded that the alkaloids contained in *Bikukulla eximia* were of slight physiological activity.

The portion of the crude total alkaloid that did not dissolve in the hydrochloric acid weighed 0.19 gm. and represented approximately 15 per cent of the whole. This was redissolved in a little alcohol and ammonia, and on the addition of water the free alkaloid separated in fine white needle crystals. The crystals were washed on a filter with water, dried, and redissolved in alcohol, in which they were readily soluble, and purified by conversion into the chloride and recovery of the free base. As thus prepared the compound was found to have a melting point of 165° C. (uncorrected) and consisted of colorless needles, very soluble in alcohol and chloroform but insoluble in water. With gold and platinum chlorides it gave only amorphous precipitates, but with picric acid it formed a finely crystalline salt which could be recrystallized from hot alcohol and which had a melting point of 175° C. (uncorrected). The alkaloid, therefore, shows every evidence of its chemical individuality and is differentiated from other alkaloids hitherto found in this group of plants by its insoluble hydrochloric acid salt as well as by other physical properties. There is every reason, then, to believe it to be a new alkaloid, and the name eximine is proposed for it.

The physiological action of this new alkaloid was tested by dissolving a weighed quantity in very dilute acetic acid and injecting the solution subcutaneously into a white mouse. A dose of 3 mgm. produced only a slight restlessness, which soon wore off, and no further symptoms could be detected. This alkaloid, therefore, is not of a dangerously poisonous character. As the other alkaloidal contents of the plant were likewise found to be innocuous, it seems safe to conclude that *Bikukulla eximia* should not be classed as a poisonous plant.

Table 1 summarizes the chief chemical and physical charactertistics of the alkaloids of the Bikukulla group that have been reported, including eximine, the new compound that has been obtained from *B. eximia*.

TABLE 1.—*Comparison of the characteristics of previously reported alkaloids of Bikukulla species with those of B. eximia*

Alkaloid	Species in which the alkaloid occurs	Melting point (° C.)	Solubility	Form
Protopine	B. cucullaria, B. pusilla, B. formosa, B. spectabilis	206–208	Ether chloroform	Needles and prisms.
Dicentrine	B. pusilla	168–169	Hot alcohol chloroform.	Prisms.
Cucullarine	B. cucullaria	168	Ether chloroform	Do.
Eximine	B. eximia	165	Alcohol chloroform	Neeeles.
Unnamed	B. canadensis	210	Alcohol	Silky, yellow needles.
Do	B. cucullaria	235	Alcohol (insoluble)	Needles.
Do	do	215	Alcohol (soluble)	Granular.
Do	B. formosa	168.5	Alcohol (insoluble)	Yellow needles.
Do	do	142.5	Alcohol (soluble)	White needles.

Alkaloid	Color tests				Physiological action
	Concentrated H_2SO_4	Erdman's	Froede's	Concentrated HNO_3	
Protopine	Yellow	Yellow to violet	Violet	Colorless	Narcotic.
Dicentrine	Violet	Blue	Deep blue	do	Toxic.
Cucullarine	Yellow	Colorless	Green to blue	Orange	Very toxic.
Eximine	Crimson	Pale yellow	do	Brown	Nontoxic.
Unnamed	Light yellow		Green	Orange	Do.
Do	Red to brown	Red to violet	Red to violet	Red to yellow	
Do	Violet	Blue	Deep blue	Colorless	
Do	Colorless	Green	Blue green	Brown	

FIELD NOTES ON THE GENUS BIKUKULLA

Bikukulla is largely a North American group of plants, with three species in the East (*B. cucullaria*, *B. canadensis*, and *B. eximia*) and several species on the Pacific coast.

An extended field survey was made in the mountain counties of southwestern Virginia in April, 1921, and in May and June, 1923. *Bikukulla eximia* was not observed in 1921, but in 1923 it was collected at Deerfield and also on the Peaks of Otter. *B. cucullaria* and *B. canadensis* were found in abundance in many places in Giles, Bland, Tazewell, Russell, and Washington Counties. They are more abundant in these localities than anywhere else in their range, but they were even more abundant before the forests were generally removed.

A better field knowledge of the western species is being acquired with a view to obtaining material for chemical study. *Bikukulla formosa*, the first species discovered on the Pacific slope, was observed by the senior writer in a number of places in the Sierra Nevada in California, but only a few plants at a time. This species is much more abundant in the moist woods along the streams flowing into Puget Sound and is common in the vicinity of Glacier, Wash. *B. chrysantha*, one of the yellow-flowered species of California. ranges

from central to southern California and is abundant in Bull Run Canyon, Sequoia Forest, Kern County. Three other species (*B. ochroleuca*, *B. uniflora*, and *B. pauciflora*) are of less importance and as yet have not been observed in any quantity. The western form of *B. cucullaria*, considered by many botanists as a distinct species, and perhaps rightly so, should be studied chemically in comparison with the eastern species.

KEY TO NORTH AMERICAN SPECIES OF BIKUKULLA

Stems leafy, tall; flowers yellow; corolla deciduous; seeds crestless.
- Flowers sulphur yellow; outer petals spreading or recurving to the middle; widely scattered. California and southern Oregon..... 1. *B. chrysantha.*
- Flowers straw yellow or cream colored; outer petals erect or only tips spreading; Santa Ynez Mountains to the Santa Monica Mountains. 2. *B. ochroleuca.*

Stems naked; leaves basal; flowers white or pink, nodding; seeds crested.
- Rootstocks tuber bearing; racemes simple.
  - Rootstocks long, with scattered cornlike yellow tubers; flowers cordate; spurs rounded..... 3. *B. canadensis.*
  - Rootstocks much shortened; tubers at base of stem.
    - Tubers gathered in a scaly, granulated bulb, white to dark red; flowers several; sagittate; spurs long, spreading..... 4. *B. cucullaria.*
    - Tubers in a fascicle, elongate, tapering toward the base; flowers 1 or 2, outer petals long, tips recurving beyond the body; small; alpine. 5. *B. uniflora.*
- Rootstocks not tuber bearing.
  - Racemes simple; flowers 1 to 3; petals distinct, outer petals narrow, recurved; small; alpine..... 6. *B. pauciflora.*
  - Racemes compound.
    - Appalachian species; flowers oblong; crest of inner petals projecting. 7. *B. eximia.*
    - Pacific coast species; corolla ovate cordate; petals united. 8. *B. formosa.*

RELATION OF PICKING TIME TO ACETALDEHYDE CONTENT AND CORE BREAKDOWN OF BARTLETT PEARS[1]

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INTRODUCTION

In the course of studies designed to throw light upon the factors associated with the production of scald and breakdown in Bartlett pears, it was reported (*1*) [2] that acetaldehyde was formed as a product of vital activity of pear protoplasm and was present in the tissues in increasing amounts during the period of ripening. The close correlation that was found to exist between relatively high quantities of acetaldehyde in the tissues and the occurrence of scald and breakdown suggested that acetaldehyde may be instrumental in the initiation of these disorders. More recent investigations have substantiated the earlier findings and have yielded further information regarding the behavior of Bartlett pears in relation to acetaldehyde content and breakdown.

It has been observed (*2*) [3] that maturity at the time of harvest is an important factor in the keeping quality of the pear. When pears are picked late in the season breaking down at the core is more prevalent than when they are picked earlier, and in general the later the time of removal from the tree the more pronounced the disease becomes. Breakdown will also occur on pears allowed to ripen immediately upon their removal from the tree without being subjected to storage conditions. Scald, on the other hand, is more prevalent on pears picked immaturely and does not appear normally until the fruit has been in storage for some time (*2*, *4*).[4]

Magness and Ballard (*5*), studying the respiration of Bartlett pears, found that the rate of ripening seemed to parallel the carbon dioxide output, that early picked fruit respired at a lower initial rate, and that this rate was accelerated less rapidly than that of late-picked fruit from the same tree. They also stated that Bartlett pears which were ripened at temperatures around 60° F. showed a marked increase in the concentration of carbon dioxide in the tissues and a considerable decrease in oxygen.

In the light of these observations it was considered important to make a further study of the acetaldehyde content of Bartlett pears, its relation to breakdown as influenced by the time of harvesting, and the carbon dioxide and oxygen concentrations within the tissues.

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[1] Received for publication Mar. 7, 1929; issued October, 1929.

[2] Reference is made by number (italic) to "Literature cited," p. 493.

[3] DIEHL, H. C. PICKING PEARS IN RELATION TO QUALITY. 10 p. [Mimeographed.] (Circular for the use of the inspectors of the cooperative Federal and Washington State shipping-point fruit and vegetable inspection service.)

[4] See footnote 3.

MATERIALS AND METHODS

In the experiments described in this paper, only Bartlett pears were used. These were obtained from two trees which were especially selected because of their similarity in location, growth, and size of crop. The fruit was picked on four different dates to represent the entire harvest period of the Wenatchee (Wash.) district, where the fruit for the experiment was obtained: August 9, 1927, the early commercial picking date; August 17, the early to midseason maturity stage; August 26, the midseason to late-season stage; and September 12, the late stage.

The pears of each picking were divided into three equal portions and placed under the following conditions: (1) Immediately ripened at 22°–24° C.; (2) stored at 0° C. for one month before being ripened at 22°–24° C.; (3) stored at 0° C. for two months and subsequently ripened at 22°–24° C. The fruit at 0° C. was held in a modern commercial cold-storage plant maintaining a fairly constant temperature with the maximum fluctuations not exceeding 2° C. In the ripening room thermographic records showed a fairly uniform range of 22°–24° C.

Samples for chemical analysis and maturity tests were taken at the time of picking, and samples for chemical analysis at intervals throughout the ripening periods. Five pears were selected at random for acetaldehyde determination. These were cut into equal pieces and samples of tissue for analysis weighed from these portions. Seeds and carpel walls were carefully excluded from the samples, and pears showing any visible evidence of scald were discarded. Separate analyses of the intercellular gas of three to five pears were made, and the individual determinations were averaged.

CHEMICAL ANALYSES

The method employed for the determination of acetaldehyde is to be found in a recent article (*1*). Samples for carbon dioxide and oxygen analyses were taken from the pears at right angles to the long axis with a large cork borer. From these samples the intercellular gases were extracted by means of a modification of the apparatus described by Magness (*3*). The gases were collected over mercury and transferred to a Bonnier-Mangin gas-analysis apparatus.

MATURITY TESTS[1]

Possibly the most accurate and satisfactory method of determining the maturity of pears is by the use of the pressure tester, a device which measures the resistance of the flesh of the fruit to mechanical pressure in terms of pounds. The instrument used in this investigation is described in Department Circular No. 350 (*6*). Standard color charts were also used in the maturity tests.

PRESENTATION OF DATA

Data secured from the chemical analyses and the maturity tests are presented in Tables 1, 2, and 3. In these tables are given the amounts of acetaldehyde in the tissues, the percentages of carbon

[1] For these data the writer is indebted to H. C. Diehl and L. A. Fletcher, Bureau of Plant Industry, U. S. Department of Agriculture.

dioxide and oxygen in the intercellular gases, the resistance of the tissues to pressure in pounds, and the average color of the fruit at the time of harvesting and at intervals during the ripening period. In Table 1 the results are from the pears picked on the four different dates and placed immediately in the ripening room at 22°–24° C. In Tables 2 and 3 are presented the analyses of the pears after periods of one and two months, respectively, in cold storage at 0° C. and then placed in the ripening room at 22°–24° C. Samples for analysis were taken upon the removal of the pears from cold storage and at intervals during the ripening period.

TABLE 1.—*Analyses of Bartlett pears ripened at 22°–24° C. immediately after picking*

Picking date (1927)	Days in ripening room	Acetaldehyde in 100 gm. of fresh tissue	Composition of intercellular gas		Average resistance to pressure	Average color [a]
			Carbon dioxide	Oxygen		
		Milligrams	*Per cent*	*Per cent*	*Pounds*	
Aug. 9	0	0	3.0	16.2	17.6	1.0
	2	.30				
	10	1.24	9.2	17.1		
	20	11.66	15.0	14.2		
	[b] 24	14.28	5.7	17.4		
	[c] 30	13.22	[d]	[d]		
Aug. 17	0	0	3.5	15.6	16.2	1.5
	2	.35	4.4	17.1		
	12	5.39	14.2	16.0		
	16	13.82	14.9	13.9		
	[b] 22	13.79	9.9	16.4		
	[c] 28	11.00	[d]	[d]		
Aug. 26	0	0	3.8	15.0	15.4	2.0
	2	.44	6.3	18.0		
	7	.72	11.5	14.7		
	13	11.29	16.0	12.0		
	[b] 19	20.10	15.2	14.2		
	[c] 24	14.40	[d]	[d]		
Sept. 12	0	0	10.6	13.4	11.0	2.5
	2	.66	15.4	12.3		
	[b] 7	14.50	14.5	15.2		
	10	14.80	24.8	11.1		
	[e] 15	15.40	[d]	[d]		
	[f] 12	12.27				

[a] Color was determined according to the method used by Magness, Diehl, and Allen (*7, p. 3*).
[b] Slight browning about vascular and core tissues.
[c] Browning about core tissues and extending into the cortex; fruit wilted.
[d] Fruit too soft to sample.
[e] Tissues badly broken down; no wilting.
[f] Badly broken down.

TABLE 2.—*Analyses of Bartlett pears placed in cold storage at 0° C. immediately after picking, and held there for one month before being ripened at 22°–24° C.*

Picking date (1927)	Days in ripening room	Acetaldehyde in 100 gm. of fresh tissue	Composition of intercellular gas		Average resistance to pressure	Average color
			Carbon dioxide	Oxygen		
		Milligrams	*Per cent*	*Per cent*	*Pounds*	
Aug. 9	0	0.22	2.9	20.6	16.3	2
	4	1.21	14.5	12.4		
	7	6.22	15.9	12.6		
	13	8.36	16.9	14.5		
	19	10.45	32.4	6.8		
	[a] 25	10.00	18.3	13.2		
Aug. 17	0	.29	3.5	20.0	15.7	2
	4	1.32	22.1	8.6		
	6	5.39	26.8	6.0		
	12	10.23	21.8	10.8		
	[a] 18	10.56	18.1	14.5		
	[b] 24	10.01				
Aug. 26	0	.11	4.5	19.1	14.4	2.5
	4	1.54	16.9	12.4		
	9	6.27	28.6	8.7		
	[c] 14	19.58	30.4	7.5		
	[d] 18	13.53	21.1	12.5		
Sept. 12	0	4.18	5.0	21.1	11.4	4
	3	9.35	26.9	7.5		
	[c] 7	16.50	32.6	8.6		
	[c] 10	20.90	20.7	10.8		
	[d] 13	13.53	31.6	5.8		

[a] Core tissues slightly browned; slight wilting.
[b] Browning of the core and cortex tissues; badly wilted.
[c] Core and cortex tissues broken down.
[d] Badly broken down.
[e] Badly broken down; no wilting.

TABLE 3.—*Analyses of Bartlett pears placed in cold storage at 0° C. immediately after picking, and held there for two months before being ripened at 22°–24° C.*

Picking date (1927)	Days in ripening room	Acetaldehyde in 100 gm. of fresh tissue	Composition of intercellular gas		Average resistance to pressure	Average color
			Carbon dioxide	Oxygen		
		Milligrams	*Per cent*	*Per cent*	*Pounds*	
Aug. 9	0	0.20	3.7	20.0	16.7	2
	4	1.32	14.7	14.5		
	9	11.22	28.9	9.7		
	[a] 18	13.53	22.1	10.2		
	[b] 22	7.92				
Aug. 17	0	.55	6.1	19.9	14.6	2
	1		8.7	17.0		
	3	1.76	23.6	9.6		
	6	8.36	21.5	4.4		
	[a] 13	11.44	19.2	10.7		
	17	10.00	16.4	14.0		
	[b] 22	9.68	18.1	11.7		
Aug. 26	0	.55	5.9	19.2	14.5	3
	5	6.27	28.7	8.8		
	[c] 9	14.74	31.2	9.5		
	[d] 14	17.71	21.0	13.2		
	17	11.22	31.5	8.0		
Sept. 12	[c] 0	11.66	6.6	19.9	11.0	4
	2	13.97	14.1	13.5		
	4	16.61	15.6	15.6		
	6	19.25	27.5	10.3		
	[d] 9	21.45	13.0	15.0		
	11	23.94	18.3	12.0		
	[a] 13	19.91	17.0	10.8		
	15	13.85	17.1	7.4		

[a] Slight browning of the core tissues; slightly wilted.
[b] Badly wilted and broken down.
[c] Core tissues broken down; no wilting.
[d] Core and cortex tissues badly broken down.
[e] All tissues completely broken down.

BREAKDOWN IN RELATION TO MATURITY

From the data presented in Tables 1 to 3 it is apparent that the stage of maturity at the time of harvesting has a decided influence upon the subsequent keeping quality of Bartlett pears. This is reflected in the length of time elapsing from the date they were removed from the tree until they showed the first evidence of breakdown. In Table 4 there are shown the number of days required to produce breakdown in the various pickings when the fruit was ripened at 22°–24° C. As evidenced there, the earlier the pears were picked the longer was the time required for breakdown to occur. This was true not only for the pears ripened immediately after picking, but also for those ripened after storage.

TABLE 4.—*Number of days required for Bartlett pears to show first evidence of breakdown when ripened at 22°–24° C.*

Picking date (1927)	Ripened immediately	Ripened after being held in cold storage for—	
		1 month	2 months
	Days	*Days*	*Days*
Aug. 9	24	25	18
Aug. 17	22	18	13
Aug. 26	10	14	9
Sept. 12	7	7	(a)

a Pears showed slight core breakdown when removed from cold storage.

These results agree with the findings of Hartman (*2*), Diehl,[6] and others, who contend that core breakdown may not be a commercial factor if proper attention is paid to maturity at the time of harvest. Diehl[6] states that, in the Northwest, Bartlett pears picked at a pressure ranging from 20 to 16 pounds have given a product of good shipping character and acceptable quality when ripened, but if picked below 16 pounds there is danger of core breakdown. Evidence obtained in the present investigation confirms this observation.

One of the physical factors which may play an important rôle in the relation between breakdown and maturity is wilting. High air humidity unquestionably retards wilting, but in these experiments, where all the lots were held under comparable conditions, the later picked fruit showed little or no wilting, while that picked early was found to show excessive wilting when ripened at 22°–24° C.

RELATION OF ACETALDEHYDE TO BREAKDOWN

In a previous paper (*1*) it was stated that acetaldehyde is a product of protoplasmic activity during the ripening period of Bartlett pears, and that comparatively large quantities of this substance are present whenever the pears exhibit scald or breakdown. In the present study the original conclusions have been confirmed so far as breakdown is concerned. In this investigation attention was directed to breakdown only, for, as has already been stated, pears ripened immediately after removal from the tree do not normally develop scald, and it

[6] See footnote 3.

was considered important in this work to include aldehyde development in pears that had not been subjected to cold-storage conditions. Therefore, for the sake of strict interpretation, any pears subsequently developing scald were discarded.

As shown in Table 1, pears analyzed immediately upon removal from the tree showed no trace of acetaldehyde in any of the pickings. However, after a period of two days in the ripening room, the tissues contained distinct quantities. From this time on there was an increase of aldehyde, reaching a maximum about the time breakdown was evident, as determined by browning of the cells.

In Tables 2 and 3 analyses are given at the ends of 1-month and 2-month storage periods, respectively. It can be seen that during storage at 0° C. acetaldehyde accumulated in all lots. In the fourth picking the aldehyde content was distinctly greater than in the earlier

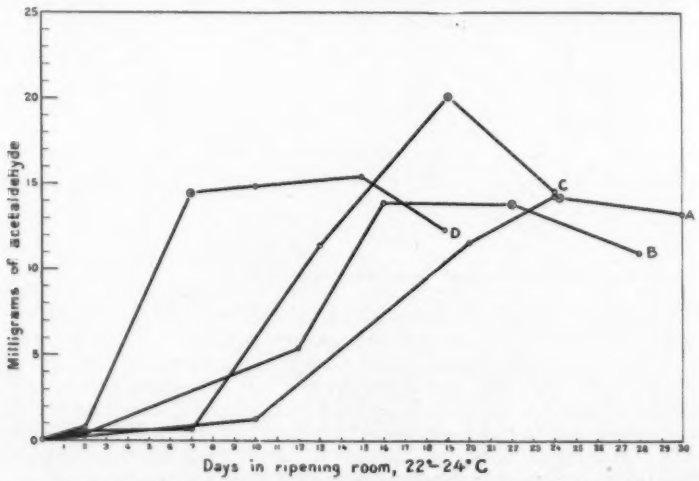

FIGURE 1.—Progress of acetaldehyde accumulation in Bartlett pears ripened at 22°–24° C. immediately after picking. Fruit was analyzed at time of picking and at intervals during the ripening period. Curve A represents acetaldehyde content of fruit picked August 9; B, August 17; C, August 26; and D, September 12

picked fruit. At the end of two months' storage the late picked pears began to show evidences of breakdown, and in these pears the concentration of aldehyde was high, even while they were in cold storage. In these cold-storage lots, as in the immediately ripened lots, aldehyde accumulation took place within the tissues when the fruit was ripened at 22°–24° C. and reached comparatively high concentrations when breakdown occurred.

A comparison of the aldehyde data of Tables 1, 2, and 3 is shown graphically in Figures 1, 2, and 3, respectively. In each of the figures, curves A, B, C, and D represent the progress of acetaldehyde accumulation through the ripening period of pears picked August 9, August 17, August 26, and September 12, respectively, from the time they were removed from the tree or from cold storage until complete breaking down of the tissues occurred. The point at which breakdown was first evident is indicated on each curve by an encircled x.

Graphic illustrations are here presented of the relation existing between the concentration of acetaldehyde within the tissues of ripening pears and the occurrence of breakdown as influenced by the maturity of the fruit at the time of harvest. A striking feature of these curves is the acetaldehyde increment as influenced by the time of picking. In general, the later the fruit was picked the more rapid was the accumulation of acetaldehyde. This is especially true of the two later pickings as illustrated by curves C and D. In the earlier pickings, curves A and B, the aldehyde values tend to merge and overlap after the cold-storage periods.

The longer period required for breakdown to appear in the early-picked pears, and the comparatively smaller amounts of acetaldehyde present in the tissues at the time of breakdown in these early picked lots, apparently carry some special significance. This condition

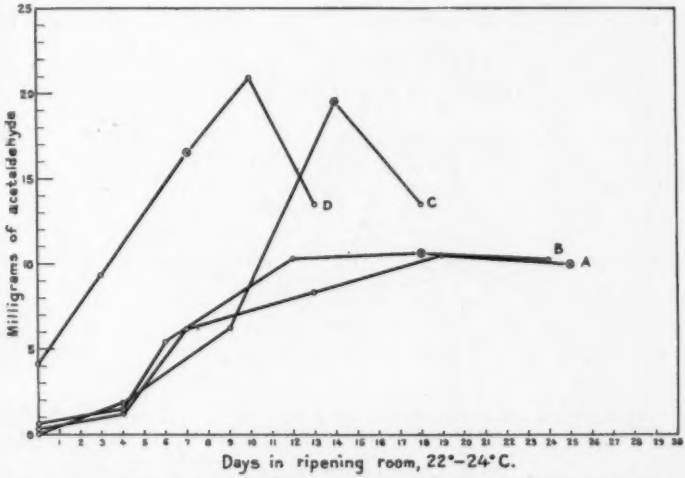

FIGURE 2.—Progress of acetaldehyde accumulation in Bartlett pears placed in cold storage at 0° C. immediately after picking, and held there for one month before being ripened at 22°–24° C. Fruit was analyzed immediately upon removal from cold storage and at intervals during the ripening period. Curve A represents acetaldehyde content of fruit picked August 9; B, August 17; C, August 26; and D, September 12

exists mainly in the fruit ripened after being held in cold storage, but it is also found to a lesser degree in that ripened immediately after harvesting. It would seem, therefore, that, if acetaldehyde is a causative agent in the production of breakdown, the concentrations of this substance would be present in the tissues in equal amounts when breakdown occurred, regardless of any of the other attending factors. This, however, is not the case, and it is evident that some factor or group of factors must be responsible for the higher concentrations of aldehyde in the late picked pears. Two hypotheses are here suggested to account for this difference in behavior. First, as the pear matures on the tree, the mechanism responsible for the production of acetaldehyde is more highly developed than in immature fruit. There is evidence to show that acetaldehyde in apple tissues is the product of a zymase system (*8*), and such an enzyme system may occur in

pears. In fact, results obtained during the course of this and other investigations indicate that this is the case. If zymasic enzymes are produced or activated during ripening, the greater aldehyde concentration in the late picked lots could thus be accounted for.

The second hypothesis is based on the physical changes that occur in the tissue structures as maturity advances. In Table 1, 2, and 3 it is noted that the early picked pears showed considerable wilting while in the ripening rooms. Certain differences in skin structure are probably of special importance in connection with this wilting. The development of corky cells in the lenticels and the accumulation of waxlike substances on the surface of the skin are no doubt responsible for the greater water-holding power of the late picked fruit. The immature or early picked pears, because of the lack of this skin development, gives up moisture more readily and in addition probably

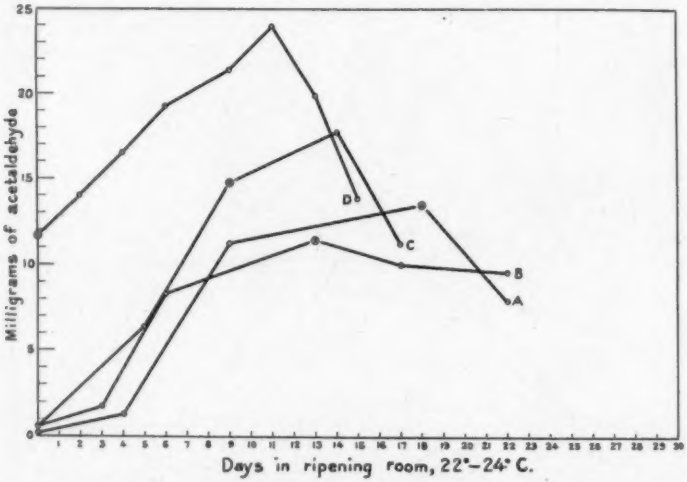

FIGURE 3.—Progress of acetaldehyde accumulation in Bartlett pears placed in cold storage at 0° C. immediately after picking, and held there for two months before being ripened at 22°–24° C. Fruit was analyzed immediately upon removal from cold storage and at intervals during the ripening period. Curve A represents acetaldehyde content of fruit picked August 9; B, August 17; C, August 26; and D, September 12

offer less resistance to gaseous exchange. It is possible that the tissues of mature fruit, which are more capable of retaining moisture, present a better anaerobic environment for zymasic activity and hence have a greater aldehyde production. It is also possible that the immature fruit may allow the gaseous aldehyde to escape more readily because of the more permeable nature of their outer tissues.

If there is an outward movement of acetaldehyde in immature pears the prevalence of scald in early picked fruit may be accounted for by the continued exposure of the skin cells to the escaping aldehyde, or these tissues may be more susceptible to injury than the cells of the cortex. The fact that some fruits that do not show tissue browning contain equal or higher concentrations of aldehyde than others that do show injury may be the result of the ability of certain cells to resist the toxic effects of the aldehyde. It is possible that

exposure to slight concentrations for a time may develop a temporary immunity in certain tissues.

These factors either separately or in combination may account for the variations that occur in the acetaldehyde content of the early picked and late picked pears. Possibly the greatest limitation in the interpretation of the production and fate of acetaldehyde is the lack of quantitative measurements of the aldehyde given off by the ripening pears to parallel the estimation of residual aldehyde present in the tissues. A study of this character should present a clearer conception of the relations existing between the above-mentioned factors and their influence upon acetaldehyde economy in pear tissues. Methods necessary to carry out this type of investigation are now being developed.

LOSS OF ALDEHYDE IN BROWNED TISSUES

It was previously reported (*1*) that after complete browning of the cells occurred, the concentration of acetaldehyde in the browned tissues showed a decided falling off, and it was concluded that the injured or dead cells were either incapable of producing acetaldehyde or unable to retain it.

This loss of aldehyde from the browned tissues was further demonstrated in the present study. Examination of the data shows a marked decrease of aldehyde following progressive injury and apparent death of the cells. The critical stage at which this loss of aldehyde becomes evident seems to vary with the maturity of the pears at the time of harvest. In the early picked lots, aldehyde began to decrease soon after the appearance of breakdown. However, in the late picked pears, aldehyde production apparently did not cease until some time after breakdown was first observed, and during this period injury continued until complete breakdown of all the tissues occurred.

CARBON DIOXIDE AND OXYGEN IN THE INTERCELLULAR ATMOSPHERE OF PEARS

Since acetaldehyde production appears to be a product of the respiratory mechanism, a measure of other substances of like origin is desirable for a broader interpretation of the process. Plant physiologists have long used the output of carbon dioxide as an index of respiratory activity; in fact, some abnormal conditions existing in fruit, including pear breakdown, have been attributed to a killing of the cells as a result of an increased concentration of carbon dioxide or of an insufficient oxygen supply.

The processes associated with the life of a cell are no doubt governed to a large extent by the gaseous medium surrounding it. Therefore, a measure of the carbon dioxide and oxygen in immediate contact with the tissues may be of more value in this study than the gross output.

In Tables 1, 2, and 3 are to be found the percentages of carbon dioxide and oxygen present in the intercellular atmosphere of pears under the conditions described. In general, with the ripening of the pears at 22°–24° C., there occurred an increase of carbon dioxide which was accompanied by a decrease of oxygen. The initial percentages of carbon dioxide in the early picked fruit were lower than

those in the later picked fruit, and the rate of accumulation was less rapid. This held true not only for the immediately ripened fruit, but also for pears ripened at 22°–24° C. after one and two months in cold storage. This is in agreement with the findings of Magness and Ballard (*5*) in their measurements of the carbon dioxide given off by Bartlett pears following both early and late picking.

In comparing fruits picked on the same date, it was found that those held in cold storage before ripening at 22°–24° C. contained higher percentages of carbon dioxide and the rate of accumulation accelerated more rapidly than those placed at 22°–24° C. immediately after harvesting.

The highest percentage of carbon dioxide in a given lot of fruit was usually found in the tissues some time prior to the first appearance of core breakdown. This is in contrast with the aldehyde content, which was found to be highest at the time browning of the tissues occurred. This fact may be of importance in establishing a relation of carbon dioxide concentration within the tissues to the occurrence of breakdown, but, since the highest concentrations of carbon dioxide occur in advance of the appearance of breakdown, it is not likely that this is the active causal agent in producing breakdown in pears. Moreover, in Table 3, it will be noticed that the pears picked on September 12 showed slight breakdown when removed from storage. The acetaldehyde concentration of these fruits, analyzed immediately upon removal, was comparatively high, whereas the carbon dioxide percentage was as low as that of the normal fruit of the earlier pickings. These data, therefore, make it improbable that carbon dioxide alone is the direct agent responsible for the breaking down of the tissues. However, from the work of Thomas (*8*) it is known that the concentration of carbon dioxide in apple tissues exerts a decided influence upon the respiratory mechanism. He found that carbon dioxide in the presence of abundant oxygen may cause the respiration of apple cells to be changed to a zymasic or anaerobic type and that acetaldehyde is a product of this type of anaerobic respiration.

Examination of the oxygen values indicates that no true carbon dioxide-oxygen ratio exists in the intercellular atmosphere as ripening progresses. In some cases high percentages of oxygen can be found in the tissues, though the carbon dioxide in the ripe pears ranged from 20 to more than 30 per cent. This strongly indicates that the respiration of Bartlett pears may be in part intramolecular in nature.

The evidence secured in this investigation indicates that the rôle of carbon dioxide and oxygen in the production of core breakdown in Bartlett pears is in the establishment of a critical environment necessary for the production of acetaldehyde.

SUMMARY

A study has been made of the acetaldehyde content of Bartlett pears, its relation to core breakdown as influenced by the time of harvesting, and the carbon dioxide and oxygen concentrations within the tissues.

It was found that the length of the period elapsing from the time the pears were picked until core breakdown occurred depended upon

the maturity of the fruit when harvested. Pears picked at an advanced stage of maturity broke down at a more rapid rate than the earlier picked fruit.

No trace of acetaldehyde could be found in pears analyzed immediately after removal from the tree, regardless of the stage of maturity. After two days in the ripening room at 22°–24° C. the tissues contained distinct quantities of aldehyde, and from this time on there was an increase, the highest concentration being reached about the time breakdown occurred.

It has been demonstrated that acetaldehyde accumulation was more rapid in the late picked pears than in the early picked and less mature fruits.

The initial percentages of carbon dioxide in the intercellular gases of the early picked fruit were lower than those in the late picked fruit, and the rate of accumulation was less rapid. The maximum carbon dioxide content of the intercellular gas was usually present some time before the first visible indication of breakdown.

In general, with an increase of carbon dioxide there was a corresponding decrease of oxygen as the pears ripened. However, in some cases fairly high percentages of oxygen accompanied high percentages of carbon dioxide. This indicates that the respiration of Bartlett pears may be in part intramolecular, which is in agreement with suggestions made by Magness and Ballard (*5*).

The results of this study indicate that the relation of carbon dioxide to the production of core breakdown in Bartlett pears is in the establishment of optimum conditions for the production of acetaldehyde.

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STARCHLIKE RADIATE CRYSTALS PRODUCED BY BACTERIUM MARGINATUM IN STARCH MEDIA[1]

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INTRODUCTION

Bacterium marginatum L. McC.[2] is a plant pathogene rather widely distributed in the United States and Canada, and it has also been reported from Europe. It causes a destructive disease on both the foliage and the corms of gladioli. This organism when grown on potato-dextrose agar or other suitable media produces in great abundance beautiful spherocrystals (fig. 1) which become blue when treated with iodine solution. In most cultures the crystals are 15 to 35 μ in diameter, but some of 140 μ have been found. They are apparently composed of needlelike parts radiating from a central point. Often there are two centers, and a double form results. (Fig. 1, B.) When broken by pressure on the cover glass they split into irregular triangular and needlelike parts.

In spite of the numerous radiating lines, the crystals are transparent. By polarized light they are faintly luminous, and the larger forms show a dark cross with the lines intersecting at right angles in the center. Some crystals show, in addition to the cross, several alternate light and dark concentric circles extending from near the center to the circumference, others have irregular or broken concentric light and dark markings, and still others show no trace of such markings. These concentric circles became invisible when a selenite plate was placed between the Nicols. Other crystals of normal appearance, but developed in a different medium, showed the dark cross less distinctly and none of the concentric marking. With a selenite plate these crystals showed two yellow and two blue-violet sectors. Still other crystals examined by C. L. Alsberg, of the Food Research Institute at Stanford University, Calif., showed "a very faint cross" and with a selenite plate between the Nicols "an alternate blue and yellow concentric-circle arrangement."

Schardinger[3,4] discovered a thermophilic bacillus, *B. macerans*, which converted a considerable amount of the starch in a culture medium into a crystalline material which he first (1903) designated as "Krystallisierte Dextrine" and later (1909) as "Krystallisierte Amylodextrin." Other workers have studied these substances, which are now generally known as crystalline amyloses.

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[1] Received for publication Feb. 28, 1929; issued October, 1929.

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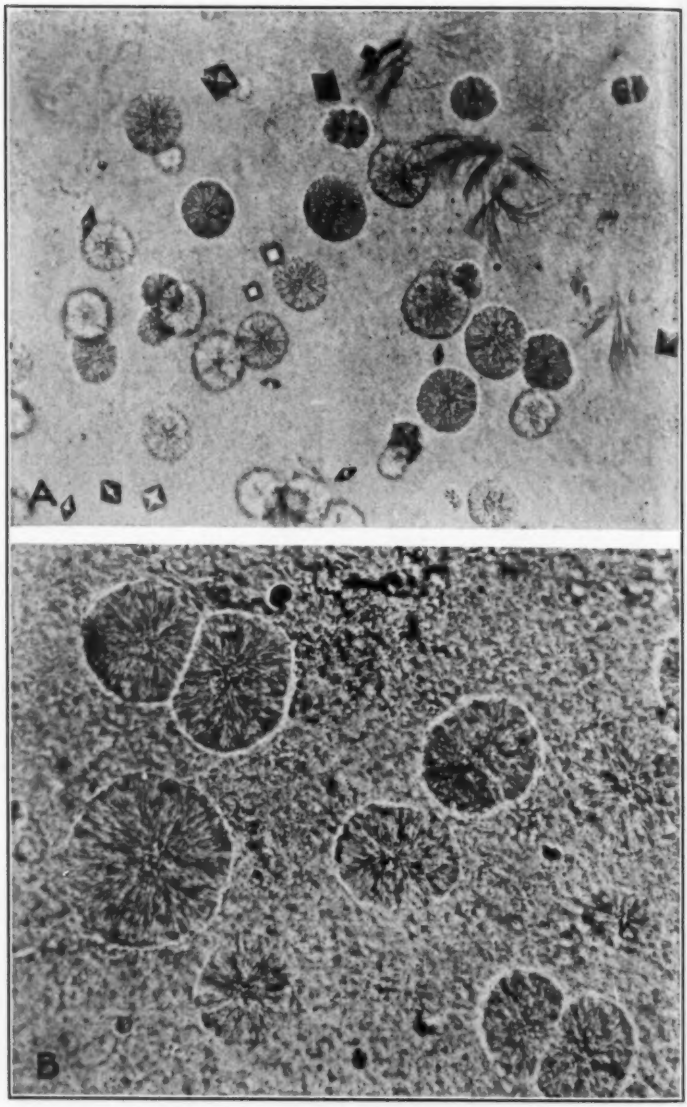

FIGURE 1.—A, Spherocrystals and also several rectangular crystals of calcium oxalate and tufts of slender, flexible crystals(?). × 250. B, Spherocrystals. × 500

The crystalline substances produced by *Bacillus macerans*, as described by Schardinger, are in form unlike those produced by *Bacterium marginatum*. However, Schardinger seems to have made microscopic examinations of his material only after various processes of filtering, neutralizing, and evaporation, which may have changed the form of the crystals. The morphological and cultural characters of *B. macerans* as given by Schardinger are very unlike those of *Bact. marginatum*.

Van de Sande-Bakhuyzen [5] pointed out that starch grains are built up of radiating needles. In a later paper [6] he demonstrated that under certain conditions spherocrystals are formed in an amylose solution which was obtained by grinding and then washing and centrifuging starch grains in cold water. The crystals so obtained are composed of several or numerous radiating needles. An alcoholic solution of iodine colors them brown to purple.

By heating a 10 per cent starch paste and then cooling it slowly, Beijerinck [7] obtained a crystalline deposit consisting of fine needles either isolated or in groups of various shapes. These he considered crystallized starch on account of their behavior toward diastase and chemical reagents.

CULTURE MEDIA

The crystals formed by *Bacterium marginatum* have been found only in culture media containing starch and a sugar or an alcohol. Potato agar with 2 per cent of dextrose [8] has been most commonly used, but a 2 per cent solution of pure starch in a synthetic solution has been successfully substituted for the potato broth. Saccharose, lactose, maltose, galactose, mannitol, and glycerol have each been successfully substituted for the dextrose.

The crystals form in either solid or liquid media. In solid media they are found in the medium, not in the layer of bacterial growth. In liquid media most of the crystals are found in the thick, almost viscid surface growth.

The crystals develop in the cultures during or just following an acid fermentation (without development of gas) and destruction of the sugar. Under favorable conditions the crystal formation, starting in the medium immediately adjoining the bacterial growth, proceeds into all parts. Crystals have been found in cultures on the third day after inoculation, and by the sixth or seventh day they are usually abundant in the upper half of the medium. They have been found in agar tubes 45 mm. below the bacterial growth and in plates 70 mm. from the nearest colony. They are apparently equally numerous (500 to 800 to the square millimeter of agar) in all parts of the culture. In agar cultures the crystals retain their structure and characteristic reaction with iodine solution in old (some 5 years old) dry cultures.

As the acid fermentation proceeds the slightly cloudy, opalescent potato-dextrose agar becomes more transparent. A cloudy line (due to closely packed, tiny, irregular crystals) usually divides the trans-

[5] VAN DE SANDE-BAKHUYZEN, H. L. THE STRUCTURE OF STARCH GRAINS OF WHEAT GROWN UNDER CONSTANT CONDITIONS. Soc. Expt. Biol. and Med. Proc. 23: 302-305. 1926.

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[7] BEIJERINCK, M. W. "CRYSTALLIZED STARCH." K. Akad. Wetensch. Amsterdam, Proc. Sec. Sci. 18: 305-309, illus. 1916.

[8] Potato, 500 gm., sliced and steamed, in 1,000 c. c. distilled water, strained; 1½ per cent agar added; steamed again, filtered; 2 per cent dextrose added; tubed, and sterilized by autoclaving.

parent fermented part from the unfermented, the line advancing as the fermentation proceeds. (Fig. 2, A and B.) The spherocrystals are abundant in the cleared, fermented area; they are occasionally found in or just beyond the cloudy line, but are entirely lacking in the unfermented part. Tests made with Fehling's solution at the close of the fermentation period show that all the sugar has disappeared, while the starch content seems unchanged. The tests for starch have been made in only a crude way by adding iodine solution and comparing with checks. In most cultures there seems to be no difference in the starch reaction between cultures and checks. In others there is apparently less starch in the cultures. More exact methods will be necessary to decide how much starch, if any, has been changed. If peptone is added to the culture medium the production of the crystals is apparently hindered, but not prevented, and the sugar is less completely destroyed.

The exact conditions and media for the best production of these crystals have not yet been determined. The potato-dextrose agar is not always suitable. Sometimes there are variations in parallel cultures of the same strain of the bacteria grown on the same medium. One tube may have numerous crystals and others few or none. A few cultures have been observed with crystals in only certain areas. Sometimes an increased amount of sugar favored and at other times seemed to hinder crystal production.

The natural pH value of the potato-dextrose medium varies from 5.2 to 6.6. By adjustment, values from 4.8 to 7.9 have been made. While the bacteria grew on all of these, the values from 5.2 to 6.6 appeared most favorable. Regardless of the original pH of the medium, the first reaction to the bacterial growth is acid. The peak of the acid reaction is 4.2 to 4.4, after which there is a change toward the alkaline, sometimes reaching 8.4

The best results up to the present both for numbers and size of the crystals were secured with a soft agar medium of pH 5.4 (potassium biphosphate, 0.2 gm.; asparagine, 1.5 gm.; dextrose, 5 gm.; galactose, 5 gm.; potato starch, 10 gm.; water, 500 c. c.; agar, 2.5 gm.). A liquid medium of pH 5.2 (potassium biphosphate, 0.2 gm.; potassium chloride, 0.05 gm.; magnesium sulphate, 0.05 gm.; calcium sulphate, 0.01 gm.; asparagine, 3 gm.; dextrose, 20 gm.; corn starch, 20 gm.; trace of iron; water, 1,000 c. c.) induced a heavy surface growth of bacteria, and numerous crystals were present three weeks after inoculation. Three months later the crystals were comparatively scarce and irregular in shape but still reacted typically with iodine solution. Another agar of pH 6.6 (potassium nitrate, 0.04 gm.; dextrose, 4 gm.; soluble starch, 2 gm.; agar, 2 gm.; water, 200 c. c.) was not favorable for the bacterial growth, but some of the starchlike crystals were produced.

Cultures were grown at temperatures ranging from 25° to 30° C.

It occasionally happens that, due to some unknown condition, the bacterial activity is limited in media supposed to be favorable. In such cases little or no fermentation occurs and no starchlike crystals develop; or some fermentation may occur without the formation of crystals.

A rough type of colony that occasionally appears in plate cultures of the normally smooth-surfaced colonies of *Bacterium marginatum* also produces the starchlike crystals.

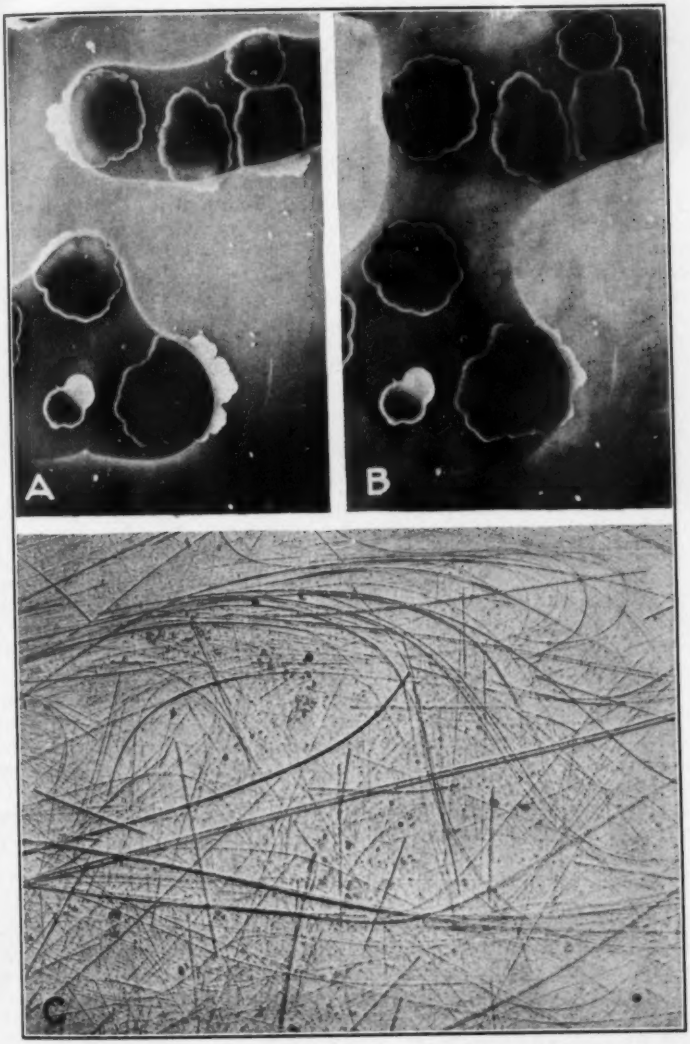

FIGURE 2.—A, Colonies of *Bacterium marginatum* in potato-dextrose agar, showing the cleared area surrounding the colonies. × 1. B, Same colonies 24 hours later, showing increased size of the cleared area. × 1. C, Long, flexible crystals(?) from potato-dextrose agar cultures of *Bact. marginatum*. × 250

All of the 20 or more strains of *Bacterium marginatum* used in the experiments have produced these starchlike crystals, but some strains have more readily and regularly than others produced crystals of good size. Some strains which formerly produced the crystals now fail to do so.

A number of other species of bacteria have been grown in parallel cultures with *Bacterium marginatum*, but none of them produced the type of crystal found so abundantly in the *Bacterium marginatum* cultures.

TEMPERATURE AND CHEMICAL TESTS

Because these crystals react like starch with iodine solution and are produced only in the presence of starch, it seems probable that they are some form of starch; but as no way has yet been found to separate the crystals from the agar of solid cultures or from the bacterial slime of liquid cultures, their exact chemical nature is unknown. In the following tests, agar containing typical crystals was crushed to a paste and the whole mass treated together.

The crystals do not dissolve in cold water, but after several days in it they become very transparent and sometimes invisible until they are stained with iodine. In water at 90° to 100° C. they dissolve quickly, but they withstand water at 80° for at least one hour. Alcohol, both ethyl and amyl, 50 to 95 per cent, has no effect on them in three hours. There is no effect from ether, chloroform, acetone, saturated solution of sodium phosphate, acetic acid (1, 10, 50, and 100 per cent), ammonia (concentrated), or 20 per cent picric acid. Alcohol (90 per cent) plus a small amount of sulphuric acid has no effect in two hours, but in 50 per cent sulphuric acid in alcohol the crystals fade away, without gas formation, in one minute or less. In iodine solution the crystals become pale blue to black, depending on the strength of the solution and also on the permeability of the surrounding media. Millon's reagent, cold for 15 minutes, has no effect, but when heated slightly the crystals dissolve without any trace of color reaction. In 5 per cent sodium hydroxide the crystals dissolve. After 20 hours in hydrogen dioxide the crystals are cracked and larger. After 12 hours in a strong solution of chloral hydrate there is no visible change in the structure of the crystals. In a 5 per cent diastase solution, at 35° to 37° C., the crystals disappear in one and one-half hours or less.

ASSOCIATED CRYSTALS

While the starchlike crystal is the most interesting crystal formed in cultures of *Bacterium marginatum*, there are several other types. Most frequently seen are those of calcium oxalate (fig. 1, A), either as small granules or as large 8-sided forms up to 75 μ in size. These occur not only in media suitable for starchlike crystals but also in beef agar, beef bouillon, plain potato broth, and 2 per cent peptone solution.

In potato-dextrose agar cultures there are found some extremely long and slender hyaline forms which were at first supposed to be crystals. There is one record of some that polarized light. It is more likely that they are some sort of gummy substance. The diameter varies from 0.5 to 1.5 μ, usually uniform throughout the

whole length, which is 10 to 700 μ. In cross section they are not round, but square or rectangular, the side walls being definitely at right angles. The longer forms are very flexible. They will bend almost double but break over a sharp angle. Often a number of these slender forms seem to originate at a common center. (Fig. 1, A.) Pressure on the cover glass usually causes them to flatten, some of them to 10 or more times their original width, and occasionally irregular enlargements form at the ends. Parallel close-lying threads sometimes coalesce, and eventually with continued pressure these crystals (?) disappear, seeming to melt and leave no trace. No water or other mounting medium was used. In cultures 2 to 3 months old these crystals (?) were still flexible, but instead of flattening or dissolving under pressure, they broke into short pieces.

Besides the preceding types, other less characteristic crystals are found, mostly in old cultures. These are irregularly spherical forms resembling hailstones, frost crystals, and rough-branched coral. Hollow brittle spheres 1 to 1.5 mm. in diameter occasionally appear in milk cultures. Their walls seem composed of minute crystals and some amorphous material. They do not dissolve in water or in 10 per cent potassium hydroxide. Alcohol, chloroform, ether, ammonia, acetone, and xylol have no effect on them, but they dissolve in dilute acetic, hydrochloric, or sulphuric acid.

SUMMARY

This paper describes a starchlike crystal which forms in cultures of *Bacterium marginatum* when starch is a constituent of the medium. These spherocrystals, up to 140 μ in diameter, are produced in great numbers. They are composed of needlelike or threadlike parts radiating from the center. They become blue when treated with iodine solution, and by polarized light the larger crystals show dark cross marks.

The chemical nature of these crystals has not been determined, because as yet no way has been found to separate them from the culture medium.

INFLUENCE OF BACTERIOPHAGE ON BACTERIUM TUMEFACIENS, AND SOME POTENTIAL STUDIES OF FILTRATES [1]

By NELLIE A. BROWN, *Associate Pathologist*, and AGNES J. QUIRK, *Senior Scientific Aid, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

While making potentiometer studies of the juices of 50 different species of healthy plants as compared with the juices of tumors produced on the plants by *Bacterium tumefaciens* Sm. and Town., the junior writer was impressed by the frequent reversal of the relative magnitudes of the pH values in the various juices after they had oxidized from one to five days. It was observed that whereas the juice of the normal sugar beet immediately after crushing might be pH 5.9 and that of the crushed tumor 6.3, in two days the juice of the normal sugar beet would be 6.2 and that of the tumor 4.8. It occurred to her that this oxidized tumor juice might influence the organism producing the tumor and that by growing the organism in a suitable medium, along with the oxidized tumor juice which had reversed its pH relative to the pH of the normal juice, there might be obtained a culture that had lost its power to infect.

Two cultures of *Bacterium tumefaciens* were accordingly exposed to different dilutions of the oxidized juice from crushed Ricinus tumors, and after the cultures had been incubated for a few days young Ricinus plants were inoculated with them. At the same time other Ricinus plants were inoculated with control cultures. Although neither treated culture became inactivated as was expected, one produced more rapidly forming and larger tumors and the other more slowly forming and smaller tumors than the controls. Recognizing that something besides oxidation with accompanying pH change had entered into these results, and believing that it was the principle of bacteriophagy, the junior writer began the work which developed into the joint investigation here reported.

REVIEW OF LITERATURE

The isolation from diseased plants of a lytic and inhibiting principle, called by D'Hérelle (*3*) [2] the bacteriophage, has not been carried on so extensively by plant investigators as by animal research workers. The earliest plant investigators who succeeded in isolating lytic principles from nodules of leguminous plants, from roots and stems of legumes, and from garden and field soil were Gerretsen, Gryns, Sack, and Söhngen (*2*). Mallmann and Hemstreet (*7*) isolated an inhibiting substance from a rotted cabbage, but did not demonstrate actual lysis. Coons and Kotila (*1*) isolated from rotted carrot, river water,

[1] Received for publication Mar. 13, 1929; issued October, 1929.
[2] Reference is made by number (italic) to "Literature cited," p. 529.

and soil the bacteriophage which produced inhibition of growth of various bacteria when used in high dilutions and definite lysis when used in stronger concentrations.

In Russia, Israilsky (Izrailsky) (*5*, *6*) demonstrated the bacteriophage in plant tumors produced by *Bacterium tumefaciens*. From nine different isolations from tumors on sugar beets he succeeded in getting two strains which were dissolved by the bacteriophage. In his later work (*6*) he studied the physical-chemical nature of the normal-plant and tumor juice along with that of *Bact. tumefaciens* in culture and concluded that the disappearance of *Bact. tumefaciens* from plant galls must be attributed to the action of a bacteriophage and not to the formation of acid in the galls. He also treated roots, stems, and seeds with the bacteriophage before inoculating them with *Bact. tumefaciens* and found that the bacteriophage reduced appreciably the percentage of infection. He stated, however, that a great many more experiments were necessary before the prophylactic action of the bacteriophage is unquestionably established.

PRELIMINARY EXPERIMENTS

In the preliminary plant inoculations made by the junior writer tumors of two distinct types were produced—one more rapidly growing and larger in size than the control tumors, and the other slow in appearing and smaller in size than the controls. The cultures were made as follows:

Good-sized Ricinus tumors were crushed well and the juice was extracted and allowed to oxidize three days, in which time the pH changed from 5.6 to 5.0. The juice was filtered through paper, and dilutions in beef bouillon of pH 7.0 were carried to about 1 : 1,400 and 1 : 28,000. The tubes were seeded with a 24-hour-old culture of *Bacterium tumefaciens* (hop strain) and left to grow two days. From these dilutions transfers were made to potato-dextrose agar, and Ricinus plants were inoculated with them two days later. Other Ricinus plants of the same age were inoculated with a potato-dextrose agar control culture 2 days old.

In 10 days the inoculations with the 1 : 1,400 dilution culture showed no indication of outgrowths, those with the control cultures had produced swellings 2 to 3 mm. in diameter, and those with the 1 : 28,000 dilution culture had produced little tumors 5 to 6 mm. in diameter. In 19 days very tiny swellings were showing from the inoculations made with the 1 : 1,400 dilution (fig. 1, A), whereas the inoculations made with the 1 : 28,000 dilution (fig. 1, B) had produced tumors twice as large as the controls, (fig. 1, C).

This comparative rate of growth kept up for over a month, the differences being quite marked. In one and one-half months the rapidly growing tumors had reached their limit, 6 cm. in diameter. This was twice the size of the controls. The retarded tumors began to grow rapidly one month after inoculation, but in two months after inoculation the largest of them was only 1.5 cm. in diameter. Very little development took place after that.

TECHNIC

Filtrates of Ricinus tumors and of sugar-beet tumors produced with the hop strain of *Bacterium tumefaciens* were added to fresh beef-bouillon cultures of the hop strain, the 2, 10, and 30 drop method

FIGURE 1.—Accelerated and retarded tumors on Ricinus. Plants inoculated January 21, 1927; photographed after 19 days. All natural size. A, Retarded and undersized tumors produced by *Bacterium tumefaciens* (hop strain) plus a low dilution of tumor juice; B, accelerated and oversized tumors produced by *Bact. tumefaciens* plus a higher dilution of tumor juice; C, normal and regular-sized tumors produced by a control (normal) culture of *Bact. tumefaciens*

of D'Hérelle (*3*) being used. These drop cultures were watched for inhibition and lysis. Several 30-drop cultures were usually made, one of which was used to make slant cultures and poured plates for the study of bacteriophage plaques. One of the 30-drop filtrate tubes had a little heavier seeding of the organism than the others, which received only a loop transfer from the young culture. The transfers to the beef bouillon were made from an 18 to 24 hour beef-bouillon culture faintly clouded or from an agar slant of the same age suspended in beef bouillon. The former was found more satisfactory, as the hop strain produces bacterial clumps, a feature not wanted in this type of work. Infusion-beef agar, pH 7.0 to 7.2, was found satisfactory for demonstrating the plaques on the plates. Beef-bouillon media of pH 6.5 to 8.3 were tried for demonstrating inhibition in the seeded filtrate cultures, but those of pH 6.7 to 6.9 were found more satisfactory. Incubation was at temperatures of 22° to 28° C.

Plates were poured from the filtrates to test their sterility, and if they proved not to be sterile this fact was noted.

When plates were poured from the seeded filtrate for the study of plaques, plates of the control cultures were also poured for comparison. Slant cultures were often made from the 2 and 10 drop seeded filtrate cultures as well as from the 30-drop ones. To seed the slants and plates, drops of cultures were carried to them in sterile pipettes, although a sterile cotton swab was sometimes used for this purpose. The seeded poured plate was found the most satisfactory of all methods to demonstrate the plaques.

Except in the case of the first experiment which demonstrated the acceleration and retardation of the growth of tumors, the juices were filtered. Chamberland L3 filters were used after the juice had first been put through filter paper or centrifuged. If one of the 30-drop seeded filtrate cultures showed inhibition or slight growth in 24 hours it was refiltered and the organism exposed again to the new filtrate. These passages of the organism with the filtrate through successive trials were carried along to increase the potency of the inhibiting substance present, in the hope that lysis would be attained. Sometimes only one and at other times several filterings and seedings were made in eight hours.

Dilutions of the tumor filtrates from 1:10 to 1:10,000,000,000 in tubes with beef bouillon seeded with the organism were also used for the study of inhibition and lysis. Control cultures were always held in the same racks for comparison.

Filtrates of normal Ricinus juice, of carrots rotted with *Bacillus carotovorus*, of normal carrots, and of sewage were also tested with the organism by both the drop and the high-dilution methods.

The strains of *Bacterium tumefaciens* tested were hop, peach, and daisy.

Plants of *Ricinus communis* (castor-oil plant) were used for the inoculations because they grow fast in the greenhouse and produce tumors quickly. The hop strain of *Bacterium tumefaciens*, which was used for most of the work, produces 100 per cent infection on Ricinus.

PHYSICAL-CHEMICAL ASPECTS OF THE FILTRATES

The study of *Bacterium tumefaciens* in culture and experiments on plants with chemical substances produced by *Bact. tumefaciens*, together with experiments limiting the intake of oxygen and so compelling the cells to manufacture the stimulus which leads to the development of hyperplasias, led Smith (*9*) to state that—

> All tumors, so far as they are due to parasites, must be assumed to be due to the chemical-physical action of the by-products of the metabolism of these parasites, just as most communicable diseases are due not to the parasites themselves, but to their toxins. * * * Diverse as are the tumors of plants due to parasites, I think that the fundamental chemical-physical phenomena underlying them are much alike, and that the differences we see, when these differences are not due wholly to varying tissue reactions, must result simply from variations in volume, and direction and continuity of the chemical-physical stimulus.

Since these statements were published, much has been added to confirm Smith's conclusions by the more direct pH, total-acid, and oxidation-potential measurements and dilution experiments on the juices of normal and tumor tissues. The striking facts revealed by these later studies are: (1) The pH of the freshly extracted juice of tumor tissue was always higher or the reaction more alkaline than that of the normal juice; (2) the titratable-acid content was greater in the tumor than in the normal juice; (3) the oxidation potential was always greater (more negative) in the freshly extracted tumor juice than in the normal juice—that is, the electrical charge carried by the gold electrode in the tumor juice was always negative (−) to that in the normal juice; (4) the electrical charges (accompanied always by a change in pH) on the gold electrodes were exactly reversed in the normal and tumor-tissue juices upon further oxidation; and (5) the change in pH produced by the growth of the organism (*Bacterium tumefaciens*) in beef bouillon cultures and the change in pH produced by *Bacillus carotovorus* when inoculated into fresh carrots progressed in the same direction (increase) as the change of pH of the juice of the normal tissue on further oxidation, rather than in the direction (decrease) of the pH changes of the juice of the tumor tissue.

The juices of the tumor are always more colloidal than the juices of normal tissue, as evidenced by the degree of filterability. The influencing factor is not precipitated by centrifugation and is active in high dilutions as an accelerator of *Bacterium tumefaciens* and in low dilutions as an inhibitory factor.

Since the substance in the plant filtrates which in the presence of molecular oxygen are apparently associated with oxidation and reduction phenomena causing changes in the pH and their oxidation potentials (with gold electrodes) behaved the same in all plants studied, and were consistently linked with the normal and tumor-tissue juices, it was thought that such potential determinations might give evidence when bacteriophage plaques could be expected on poured plates. That there was some justification for this belief is brought out by a study of Table 1 and the poured-plate and plant-inoculation experiments given later.

TABLE 1.—*Titratable-acid, pH, and oxidation-potential measurements* [a] *of different filtrates and the plaques on poured agar plates resulting from treating the cultures of Bacterium tumefaciens (hop strain) with them*

Filtrates and cultures tested	Freshly extracted filtrates and young cultures				Oxidized filtrates and old cultures		Plaques
	Initial pH	N/1 alkali required to increase pH to 8.2	Oxidation potential differences	Charge (plus or minus)	Initial pH	Charge (plus or minus)	
		C. c.	*Millivolts*				
Ricinus with mature tumor:							
Tumor	5.5	22.5	125.6	−	4.9	+	Present.
Normal	5.4	16		+	6.8	−	
Ricinus with rotted tumor:							
Tumor	6.8		103.0	−	7.1	−	Absent.
Normal	5.4			+	8.2	−	
Ricinus with dried tumor plus water:							
Tumor	6.8			−	6.8	−	Very few present.
Normal	5.5			+	8.0	−	
Sugar beet with mature tumor:							
Tumor	6.2	23.5	125.0	−	5.0	+	Present.
Normal	5.9	18		+	6.4	−	
Sugar beet with older, mature tumor:							
Tumor	6.8	25	143.2	−	5.1	+	Do.
Normal	6.3	19.5		+	6.8	−	
Carrot:							
Normal, fresh and young	6.8						
13-day-old normal, young					7.1		
Inoculated 70 days with Bacillus carotovorus.					9.6		
Bacterium tumefaciens:							
Plus juice of carrot infected with B. carotovorus; culture 1 day old.	[b] 7.1						Many present.
Plus juice of carrot infected with B. carotovorus; culture 4 days old.					6.6		
24-hour-old normal culture	6.7						Present.
1-month-old normal culture					8.2		Absent.

[a] The followingprocedure was suggested by S. F. Acree of the Bureau of Standards: Two batteries, consisting each of a saturated calomel electrode and a Hildebrand cell, were joined by a platinum wire, connecting the two calomel cells. Each Hildebrand cell contained a gold or a hydrogen electrode as desired. Usually 10 c. c. of the normal or tumor juice was used for each test. This equipment made it possible (1) to measure both the hydrogen-ion and the oxidation potentials against a standard calomel cell, or (2) to obtain directly the differences in these potentials shown by the normal and tumor extracts. The latter method was used generally with occasional checks of each sample against the calomel electrode with a type K Leeds & Northrup potentiometer. The estimation of potentials was made on expressed juice of normal and tumor tissue. The same volume of normal and tumor juice from the same plant was used for comparison in each experiment. The same environmental conditions as to temperature, gases, technic, etc., made the results more accurate as they are dependent on the simultaneous handling of both samples.

[b] This culture was split and tested 4 days later, but no plates were poured on the fourth day.

It is shown in Table 1 that the freshly extracted juice from Ricinus and sugar beet gave pH values of 5.4 to 6.3 and carried a positive (+) charge when compared to the juice of the tumor; the same juice oxidized from two to five days gave pH values progressing in the alkaline direction accompanied by a minus (−) charge. The opposite relationship of values, both as to pH and to charge carried, to those of the normal juice was obtained on the freshly extracted and oxidized tumor juice. The freshly extracted juice of a mature tumor was more alkaline than the normal juice in its initial pH values and carried a minus (−) charge, but upon oxidation for two to five days the pH values became distinctly more acid with an accompanying change of electrical charge (+). *Bacterium tumefaciens* treated with this filtrate showed numerous plaques on the poured plates. In the case of the rotted tumor juice the pH value proceeded in an alkaline direction, and in that of the dried tumor juice plus water the value remained about

stationary. It is a striking fact that from the last two filtrates, where the pH progressed in the alkaline direction or remained stationary, few or no plaques were found on the poured plates. The total acid content was found to be greater in the freshly extracted tumor juice than in the normal juice; also a definite oxidation-potential value in favor of the freshly extracted tumor juice above that of the normal juice was registered. The reversal of pH ceased at about pH 5.1 to 4.9 in the juices of the tumors from Ricinus and sugar beet.

The freshly extracted juice of young carrots gave pH values of 6.8, whereas the same juice left standing 13 days gave pH 7.1. A *Bacterium tumefaciens* culture treated with the fresh filtrate of normal carrot produced plaques. Juice of carrots 70 days after inoculation with *Bacillus carotovorus* gave pH 9.6. The pH direction taken by carrot inoculated with *B. carotovorus* is the same as that taken by *Bact. tumefaciens* in beef-infusion medium and that of the normal-carrot juice upon further oxidation.

A freshly seeded *Bacterium tumefaciens* culture treated with the filtrate of carrots inoculated with *Bacillus carotovorus* gave a value of pH 7.1. With this filtrate the plaques on poured plates were more numerous than with any of the other filtrates. This same culture held four days gave pH 6.6. Here again the pH is in the more acid direction when compared with the values of the juice from carrot inoculated with *B. carotovorus* (pH 9.6) and *Bact. tumefaciens* culture 1 day old (pH 6.7) or 1 month old (pH 8.2). At two different times a 24-hour culture of *Bact. tumefaciens*, pH 6.7, gave good plaques on poured plates. These were exceptions rather than the rule. For the range of growth of *Bact. tumefaciens* in beef-infusion medium an earlier paper (*8*) may be consulted.

Three Ricinus plants were inoculated with each of the tenfold dilution cultures when the cultures were two days old, and three other Ricinus plants were inoculated with a control culture of the same age. In nine days many of the little tumors resulting from the dilution inoculations were growing faster than the control tumors. In less than a month the tumors forming on 17 of the plants inoculated with the dilution culture were larger than the control-culture tumors, 8 were of the same size, and only 5 were slightly smaller. All of the sixth-dilution (1:1,000,000) inoculations produced smaller tumors than the controls, while all the first, fifth, eighth, and two each of the seventh and tenth dilutions produced larger tumors.

To determine whether inhibition might occur if larger quantities of the same filtrate were used in the bouillon, a series of dilutions was made with 9 parts of the filtrate to 1 part of beef bouillon. To a tube of 1 c. c. of beef bouillon 9 c. c. of filtrate was added, thus making a 9:10 dilution. Ten dilutions were made as before, the last being 9:1,000,000,000. Each was seeded with a loop of a 24-hour culture of the hop strain. Heavier growth than in the controls took place in 24 hours in all cultures except the 9:10, which had none. A heavy pellicle formed in all except the 9:10. In 48 hours, however, this culture also had what appeared to be a trace of growth. Inoculations with these 11 cultures (including the control) were made the second day into each of three Ricinus plants.

Practically the same relationship of acceleration of tumor growth and increased size of tumors over the controls was observed in the

9:10 series as in the 1:10 series. Inoculations with the first dilution, 9:10, did not produce tumors. Table 2 gives the results of the inoculations with both series.

TABLE 2.—*Results of inoculating Ricinus plants with the hop strain grown two days with filtrates of Ricinus tumors in dilutions of 1:10 to 1:10,000,000,000 and 9:10 to 9:1,000,000,000, respectively*

Dilution and period between inoculation and examination	Dilutions of 1:10 to 1:10,000,000,000				Dilutions of 9:10 to 9:1,000,000,000			
	Initial pH	Diameter of tumors on—			Initial pH	Diameter of tumors on—		
		Plant No. 1	Plant No. 2	Plant No. 3		Plant No. 1	Plant No. 2	Plant No. 3
		Mm.	*Mm.*	*Mm.*		*Mm.*	*Mm.*	*Mm.*
No. 1:								
9 days	6.8	9–12	9–12	9–12	5.3	0	0	0
1 month		20–30	20–30	20–30		0	0	0
No. 2:								
9 days	7.1	9–12	0	0	5.4	9–12	9–12	9–12
1 month		30–40	13–20	9–12		20–30	20–30	20–30
No. 3:								
9 days	6.8	9–12	9–12	9–12	7.0	9–12	9–12	6–8
1 month		30–40	13–20	13–20		20–30	20–30	9–12
No. 4:								
9 days	6.7	9–12	9–12	9–12	7.0	9–12	0	0
1 month		20–30	13–20	9–12		13–20	13–20	9–12
No. 5:								
9 days	6.9	9–12	9–12	9–12	7.1	9–12	9–12	6–8
1 month		20–30	20–30	20–30		20–30	9–12	9–12
No. 6:								
9 days	7.3	9–12	4–5	4–5	7.1	9–12	0	0
1 month		9–12	9–12	9–12		9–12	9–12	9–12
No. 7:								
9 days	6.9	9–12	0	0	6.9	9–12	9–12	6–8
1 month		20–30	20–30	13–20		9–12	9–12	20–30
No. 8:								
9 days	6.7	9–12	0	0	6.9	9–12	9–12	0
1 month		20–30	20–30	20–30		20–30	20–30	9–12
No. 9:								
9 days	7.1	9–12	0	0	7.1	9–12	0	6–8
1 month		20–30	13–20	13–20		20–30	20–30	9–12
No. 10:								
9 days	7.0	9–12	9–12	9–12	7.3	9–12	0	0
1 month		30–40	30–40	13–20		20–30	20–30	20–30
Control: *								
9 days	7.8	4–5	0	0	7.8	4–5	0	0
1 month		13–20	13–20	13–20		13–20	13–20	13–20

* Same control plants used for both dilution series.

EFFECTS OF DIFFERENT TREATMENTS ON THE HOP STRAIN

EFFECTS OF DILUTE FILTRATE OF RICINUS TUMORS ON PRODUCTION OF TUMOR

JUICE FILTERED AFTER 3-DAY OXIDATION

Filtrates of Ricinus tumor which were found to be sterile by the poured-plate method after passing through Chamberland L3 filters were studies in dilution experiments. The juice had been exposed to the air for three days before it was filtered and its pH had changed from 5.5 to 4.9. A series of tubes containing 9 c. c. of infusion-beef bouillon, pH 6.9, was treated as follows: With a sterile pipette 1 c. c. of a filtrate of a Ricinus tumor was added to the first tube and mixed thoroughly by shaking. This gave a dilution of 1:10. With another sterile pipette 1 c. c. of this dilution was added to the next tube, making the second a 1:100 dilution. The method was repeated, each tube was shaken and a sterile pipette was used for each of the

10 tubes of the series until a dilution of 1:10,000,000,000 was reached. The dilutions were each seeded with a loop of a 24-hour bouillon culture of the hop strain, for in a previous test a drop of the culture had been found to be too heavy.

In 18 to 24 hours growth was heavier than in the controls in all but the 1:10 dilution. In 2 days there was also growth in the 1:10 dilution.

Juice Filtered Immediately

The dilution series 1:10,000,000,000 was repeated with two other filtrates (pH 5.8 and 5.5) from Ricinus tumors. As unoxidized juice was desired for this experiment, both sets were filtered, diluted in series, and seeded with the hop strain the same day the tumors were cut. No retardation of growth was observed in the tubes of either set except in the 1:10 dilutions, which lasted for only about 24 hours. Both sets were well clouded in 48 hours. The other nine dilutions showed greater growth than the controls in 24 hours.

Two Ricinus plants were inoculated with each of the 10 dilutions of the second of the unoxidized sets. Two inoculations with control cultures were also made. The tumors grew more rapidly and developed to greater size in the fifth, sixth, seventh, and eighth dilution inoculations than in the controls; the tumors in the ninth and tenth dilution inoculations were the same size as control tumors; while the tumors in the first, second, third, and fourth dilution inoculations within a little more than a month were slightly smaller than those of the controls.

From these tests it appears that tumor juices filtered and used immediately and those oxidized several days before filtering and used in high dilutions with the organism have power to produce more rapidly growing and larger tumors than the control cultures; they also have power to produce larger tumors than the controls when they are inoculated into susceptible plants.

Comparative Effects of Filtrates of Bacterium tumefaciens, Normal Ricinus Plants, and Necrosed Ricinus Tumors

A 24-hour beef-bouillon flask culture of *Bacterium tumefaciens* was filtered, the filtrate was carried in tubes in dilutions from 1:10 to 1:10,000,000,000, and each dilution was inoculated with *Bact. tumefaciens* (hop strain) to see how the organism would react with a culture filtrate of itself added. In 24 hours there was slight growth in the controls but no growth in any of the dilutions. In 48 hours, however, there was growth in all 10 dilutions, and in many of them it was heavier than in the controls.

A filtrate of normal Ricinus plants and one of necrosed Ricinus tumors were carried each in 10-dilution tubes in the same way, 1:10 to 1:10,000,000,000, and each dilution was inoculated with the hop strain for comparison with the culture-filtrate dilutions. In 24 hours no growth had occurred in these two sets. In 48 hours, however, there was heavier growth in some of the dilutions with filtrate of the normal Ricinus plant than there was in the controls. There was growth equal to the controls in the cultures with necrosed Ricinus tumor filtrate. The pellicles were not so heavy as in the controls in the 1:10 and 1:100 dilutions of the latter.

Inoculations into young Ricinus plants were made when the dilution cultures were 4 days old. The plants were a little younger than is desirable for inoculating and the growing tumors killed a few of them. Many of the culture-filtrate dilutions produced tumors more rapidly than the control cultures, the normal Ricinus filtrate cultures, or the necrosed tumor filtrate cultures. The tumors were also larger than those produced by the necrosed tumor filtrate inoculations, but were not very different in size from those of the normal Ricinus tumors. Table 3 gives details of this experiment.

EFFECTS OF REPEATED FILTERINGS WITH DIFFERENT FILTRATES

Filtrates of Old and Slightly Necrosed Ricinus Tumors

The pH of the juice of 5-months-old Ricinus tumors was 6.8 before filtering and 6.2 after filtering. The pH of the juice of healthy Ricinus plants of the same age was 5.4.

Two, ten, and thirty drops of the 5-months-old tumor filtrate were added to fresh (hop strain) cultures in beef bouillon (pH 6.7). In 24 hours the growth was slight in the drop cultures but equal to that in the controls. Bacteriophage plaques were present on the drop plates, however, in two days. Refilterings of the drop cultures were made, and the tests with tube cultures and poured plates were carried through the third passage. There was still no inhibition in the third passage, and no bacteriophage plaques occurred in the drop-filtrate or the control plates.

Seven days later more of the 5-months-old Ricinus tumor juice was filtered, and *Bacterium tumefaciens* was exposed to it in the usual way. The filtrate had then changed from pH 6.8 to 7.1. A test with 2, 10, and 30 drops was made in the morning and another in the afternoon, using hop strain in beef bouillon (pH 6.7). In both sets growth of the organism was more rapid in the drop tubes than in the controls. No bacteriophage plaques were present on the drop-filtrate plates. The plates poured to test the sterility of the old tumor filtrate had contaminating but no *Bact. tumefaciens* colonies. To make a further test, the unused part of the 7-day-old tumor filtrate was filtered a second time. The filtrate was sterile, for no contaminating colonies appeared on the plates poured from it. The hop strain was again exposed to the old filtrate by the drop method to see if retardation would occur. There was none.

Table 3.—*Results of inoculating Ricinus plants with Bacterium tumefaciens (hop strain) grown four days in 1:10 to 1:10,000,000,000 dilutions of filtrates of Bacterium tumefaciens (hop strain) of normal Ricinus plants, and of necrosed Ricinus tumors, respectively*

Dilution and period between inoculation and examination	Filtrate of beef-bouillon culture of hop strain (pH 6.7)			Filtrate of normal Ricinus plants (pH 5.4)			Filtrate of necrosed Ricinus tumors (pH 6.8)		
	Initial pH	Diameter of tumors on—		Initial pH	Diameter of tumors on—		Initial pH	Diameter of tumors on—	
		Plant No. 1	Plant No. 2		Plant No. 1	Plant No. 2		Plant No. 1	Plant No. 2
1 to 10:		*Mm.*	*Mm.*		*Mm.*	*Mm.*		*Mm.*	*Mm.*
10 days	7.8	9–12	9–12	7.7	6–8	6–8	7.5	6–8	6–8
19 days		13–15	13–15		9–12	9–12		13–15	13–15
2½ months		35–45	Dead.		18–28	18–28		15–18	15–18

TABLE 3.—*Results of inoculating Ricinus plants with Bacterium tumefaciens (hop strain) grown four days in 1:10 to 1:10,000,000,000 dilutions of filtrates of Bacterium tumefaciens (hop strain) of normal Ricinus plants, and of necrosed Ricinus tumors, respectively*—Continued

Dilution and period between inoculation and examination.	Filtrate of beef-bouillon culture of hop strain (pH 6.7)			Filtrate of normal Ricinus plants (pH 5.4)			Filtrate of necrosed Ricinus tumors (pH 6.8)		
	Initial pH	Diameter of tumors on—		Initial pH	Diameter of tumors on—		Initial pH	Diameter of tumors on—	
		Plant No. 1	Plant No. 2		Plant No. 1	Plant No. 2		Plant No. 1	Plant No. 2
		Mm.	*Mm.*		*Mm.*	*Mm.*		*Mm.*	*Mm.*
1 to 100:									
10 days	7.7	9–12	9–12	7.7	6–8	6–8	7.5	6–8	6–8
19 days		13–15	13–15		9–12	9–12		13–15	13–15
2½ months		30–35	Dead.		35–45	18–28		15–18	15–18
1 to 1,000:									
10 days	7.5	9–12	9–12	7.2	6–8	6–8	8.1	6–8	6–8
19 days		13–15	13–15		6–8	6–8		9–12	9–12
2½ months		28–30	15–18		35–45	35–45		15–18	Dead.
1 to 10,000:									
10 days	7.5	9–12	9–12	7.6	6–8	6–8	8.1	6–8	6–8
19 days		9–12	9–12		9–12	9–12		13–15	9–12
2½ months		35–45	15–18		35–45	18–28		18–28	18–28
1 to 100,000:									
10 days	7.5	9–12	9–12	7.8	6–8	6–8	8.2	6–8	6–8
19 days		13–15	9–12		13–15	9–12		9–12	6–8
2½ months		35–45	30–35		35–45	18–28		18–28	15–18
1 to 1,000,000:									
10 days	7.5	9–12	9–12	7.7	6–8	6–8	8.2	4–5	4–5
19 days		13–15	13–15		13–15	9–12		9–12	9–12
2½ months		30–35	30–35		18–28	18–28		18–28	18–28
1 to 10,000,000:									
10 days	7.5	9–12	9–12	7.7	6–8	6–8	7.7	4–5	4–5
19 days		13–15	13–15		9–12	9–12		9–12	9–12
2½ months		35–45	35–45		18–28	18–28		15–18	15–18
1 to 100,000,000:									
10 days	7.5	9–12	9–12	7.6	6–8	6–8	8.0	6–8	6–8
19 days		9–12	9–12		9–12	9–12		9–12	9–12
2½ months		35–45	30–35		35–45	35–45		18–28	15–18
1 to 1,000,000,000:									
10 days	7.5	9–12	9–12	7.7	6–8	6–8	7.4	6–8	6–8
19 days		9–12	9–12		13–15	13–15		9–12	6–8
2½ months		18–28	28–30		18–28	Dead.		18–28	9–12
1 to 10,000,000,000:									
10 days	7.5	9–12	9–12	7.7	6–8	4–5	7.7	6–8	6–8
19 days		13–15	9–12		13–15	9–12		9–12	9–12
2½ months		15–18	15–18		18–28	18–28		18–28	15–18
Control: [a]									
10 days	7.3	6–8	6–8	7.3	6–8	6–8	7.3	6–8	6–8
19 days		9–12	9–12		9–12	9–12		9–12	9–12
2½ months		18–28	18–28		18–28	18–28		18–28	18–28

[a] Control culture 4 days old. The same control plant was used for each dilution series.

This time, as the filtrate was sterile, in 24 hours the growth in the filtrate tubes was equal to that in the controls but not greater. It duplicated that in the third-passage tests made when the filtrate was used immediately after the tumors were crushed. No bacteriophage plaques appeared on the plates. The direction of the pH change of the filtrate of this particular Ricinus tumor was to the more alkaline side, which is contrary to that usually found in the younger tumors.

FILTRATES OF OLD DRY RICINUS TUMORS

It was thought that an active bacteriophage might have prevented necrosis in the old Ricinus tumors, for even when 6 to 7 months old they were still sound, although more or less dry. Fifty

tumors were ground up, and the juice was extracted and filtered. The hop strain was exposed to 2, 10, and 30 drops of this filtrate, and still other tests were made with the dry tumors. Some of the mashings were left in extract and infusion-beef bouillon for 5 and 24 hours, respectively. Each solution was filtered, and the hop strain was again exposed to 2, 10, and 30 drops of the different filtrates.

None of the filtrates of the dry tumor showed inhibition, and the bacteriophage plaques on the plates accompanying each test were very few and small or there were none at all. In each test there was refiltering with accompanying exposure of the hop strain, but still no inhibition or acceleration of growth as compared to that of the controls was noted.

Filtrates of Sugar-Beet tumors

Tumors on the sugar beet were produced with the hop strain of *Bacterium tumefaciens*. They were crushed, the juice was filtered and 2, 10, and 30 drops of the filtrate were added to fresh beef-bouillon transfers of *Bact. tumefaciens*. Growth in the 2 and 10 drop cultures in 24 hours was heavier than in the control, while that in the 30-drop culture was retarded. In order to speed up the potency of the inhibiting factor, one of the 30-drop cultures was filtered and 2, 10, and 30 drops of this were added to a fresh beef-bouillon culture of *Bact. tumefaciens*. These filterings were carried through five passages, and while plaques appeared on the agar plates and slants, indicating the presence of the bacteriophage, the inhibition in the cultures lasted only 2 to 3 days. Hardened agar plates streaked with cultures made from several of the refiltered and inoculated cultures also showed the bacteriophage plaques.

Some of the plaques were transferred to fresh beef-bouillon cultures of *Bacterium tumefaciens*, but no difference was noted between the plaque-containing culture and the controls. The bacteriophage did not seem to be potent enough to cause lysis or even to retard cultures.

Ricinus plants were inoculated with the 10-drop and with one of the two 30-drop cultures of the original filtrate and organism. These inoculations produced tumors somewhat more rapidly than the controls and for about three weeks they were larger. In one month, however, the tumors in the controls equaled them in size.

A filtrate of other sugar-beet tumors (hop strain), oxidized for 12 days, in which the pH had changed from 6.2 to 5.0, was also used with *Bacterium tumefaciens*. Ten drops of the filtrate added to a transfer from a 24-hour culture of the hop strain produced a culture that had an unusual appearance. In the 10 days that it was under observation no pellicle formed, but there was a heavy precipitate in the bottom of the tube. The precipitate was not viscid as such precipitates usually are, and there was a heavy suspension of growth throughout the culture. Inoculations into Ricinus plants were made with the culture when it was 10 days old. The inhibiting factor evidently was present, for none of the Ricinus plants inoculated showed any outgrowths until after 5 weeks; then tumors appeared and developed very slowly. In 2 months they were only 1 cm. in diameter, while the control tumors were 4 cm. in diameter.

SEWAGE FILTRATE

A sewage filtrate was also used in the 2, 10, and 30 drop method with the hop strain. There were many refilterings, and the filtrate was added each time to a freshly seeded beef-bouillon culture. Retardation of growth did not occur until the third passage, and it did not last 48 hours. Filterings were continued with the use of one of the bacteriophage plaques from the third-passage plates in culture with the organism. The passages which followed produced more of the bacteriophage plaques on plates and slants, but retardation in the tube cultures was about the same. In the fifth passage of the plaque filterings growth was more rapid in the tube cultures than in the controls, and the plaques on the plates practically disappeared.

To ascertain whether this acceleration of growth in the tubes could be demonstrated in tumors on plants, Ricinus plants were inoculated with the 10 and 30 drop cultures. At the same time other Ricinus plants of the same age were inoculated with control cultures, and still others with 10 and 30 drops of sugar-beet tumor filtrate grown with the hop strain. In a week the sewage-filtrate hop inoculations showed well-defined outgrowths 4 mm. in diameter, the beet-filtrate hop inoculations showed good swellings though smaller, while the control-culture inoculations showed mere surface swellings. These differences continued for nearly three weeks, but in four weeks the control and the beet-filtrate tumors had nearly reached the size of the sewage-filtrate tumors.

EFFECTS ON THE DAISY STRAIN OF REPEATED FILTERINGS WITH FILTRATES OF SUGAR-BEET TUMORS

The attempt to isolate an inhibitory substance from filtrates of sugar-beet tumor was continued with these filtrates in company with the daisy strain of *Bacterium tumefaciens*. A feature of this strain is that it does not grow so rapidly on beef agar and in beef bouillon as does the hop strain.

In the first two passages of the sugar-beet tumor filtrate and the daisy strain there was greater growth than in the controls, and no bacteriophage plaques appeared on the plates. Slight inhibition began in the third passage.

Marked inhibition of growth in the 2, 10, 30, and 60 drop filtrate tubes inoculated with the daisy strain occurred, and bacteriophage plaques appeared on the plates of the fourth passage. A plaque from one of these plates was transferred to a slightly clouded beef-bouillon culture of daisy strain, incubated, filtered, and further passages made with the daisy strain, sometimes two and three and at other times only one in eight hours. This was continued through 24 passages. The activity of this filtrate embracing the bacteriophage plaque was marked beginning with the second passage, for there was inhibition of growth in the tube cultures, and plaques appeared on the plates from the second through the eighteenth passage. At this stage an older agar suspension was used instead of the usual 18 to 24 hour culture. With this older culture the activity of the bacteriophage seemingly was submerged, for no plaques appeared on the plates from the nineteenth to the twenty-fourth passage, when the experiment was discontinued. Neither was there a trace of inhibition of growth

in the 2, 10, 30, and 60 drop cultures of the twentieth to the twenty-fourth passage, and only slight inhibition in the nineteenth.

Partial lysis occurred in the tenth passage. Inhibition of growth continued up to the twelfth day in the 30-drop tube, then growth began, but there was only faint clouding with no formation of pellicle. Dead bacteria were in a precipitate at the bottom of the tube, and there was no motion in those alive in the suspended medium when examined in hanging drops.

Ricinus plants were inoculated with a portion of the tenth-passage inhibited culture, and other Ricinus plants were inoculated with a control culture of the same age. Tumors appeared on the control plants, but none on those inoculated with the inhibited culture. Examination of plants was made as late as two and one-half months after the inoculations.

The eleventh passage of the daisy strain with the sugar-beet tumor also showed inhibition in the 30-drop culture. Cowpeas were inoculated with this culture after growth took place, but no tumors resulted. The control inoculations gave small tumors.

Inhibition was not so marked in the twelfth to the eighteenth passages.

Some of the inhibited tenth-passage culture was filtered and treated in a series of new passages with the daisy strain. Much was expected of this filtrate, but no trace of inhibition was shown in two passages. This may have been due to the fact that an older culture of daisy was used for the initial passages, as an 18 to 24 hour culture was not available. It is quite essential to use a 24-hour or less beef-bouillon or agar culture suspension in beef bouillon.

EFFECTS OF FILTRATES OF ROTTED CARROTS ON THE HOP STRAIN OF BACTERIUM TUMEFACIENS AND OTHER ORGANISMS

When *Bacterium tumefaciens* (hop strain) was exposed to rotted-carrot filtrate and carried through a number of refilterings, the inhibition was longer than when it was carried through successive refilterings with tumor filtrates.

Sound carrots were inoculated with a pure culture of *Bacillus carotovorus* and kept in a moist chamber until a quantity of juice from the rotting roots had collected. This was filtered, and by means of poured plates it was found to be sterile. The filtrate was added to cultures of the following organisms to test their susceptibility to it: *Bacterium tumefaciens* (hop, peach, and daisy strains), *Bacillus carotovorus*, *B. mycoides*, *B. coli*, and *B. phytophthorus*. Loop transfers were made from a 24-hour beef-bouillon culture of all but *B. mycoides*, which had too heavy a growth in 24 hours, so a 5-hour culture of it was used. Transfers were made to pH 7.4 beef bouillon, and 2, 10, and 30 drops and 2 c. c. of the filtrate were added to the cultures. The hop strain of *Bact. tumefaciens* exposed to the 2 c. c. of filtrate was the only organism that showed inhibition. This inhibition lasted only three days. The effect of the filtrate continued, however, for the hop cultures did not produce pellicles, while the hop controls and the treated peach and daisy strains all had heavy pellicles.

The effect of 30 drops and 2 c. c. of the carrot filtrate on *Bacillus carotovorus*, the organism that rotted the carrot, was to produce a

slightly heavier growth than the control in 24 hours instead of inhibiting it.

Table 4 shows the effect of the carrot filtrate on the several organisms treated with it for 24 hours. In three days there was good growth in all the cultures that had shown slight growth except the hop strain. The hop strain then had slight growth in the 30-drop and 2 c. c. filtrate cultures as well as in the 2-drop and 10-drop ones.

TABLE 4.—*Effect of adding filtrate of carrots rotted with Bacillus carotovorus to cultures of seven different organisms*

Organism	Growth 24 hours after adding—				Growth in control culture after 24 hours
	2 drops	10 drops	30 drops	2 c. c.	
Bacterium tumefaciens:					
Hop strain	Slight	Slight	Questionable	None	Good.
Peach strain	Fair	Fair	Slight	Very slight	Do.
Daisy strain	Slight	do	Good	Questionable	Do.
B. carotovorus	Fair	do	Heavier than control.	Heavier than control.	Heavy.
B. mycoides	Heavy	Heavy	Fair	Fair	Do.
B. coli	do	do	do	do	Do.
B. phytophthorus	do	do	do	do	Do.

The experiment described above was repeated with the three strains of *Bacterium tumefaciens*, more of the rotted-carrot filtrate being used. Beef-bouillon tubes, pH 7.4, were seeded with a loop of a 24-hour culture of each organism, and because of the small quantity of the filtrate, only 2, 4, and 10 drops of it were added.

The daisy and peach strains showed such slight inhibition after the third passage that they were discontinued. The hop strain, on the other hand, produced marked inhibition at once, and in 48 hours only the merest trace of growth was discernible in the 10-drop tube and a faint clouding in the 2 and 4 drop tubes, while the controls had heavy growth with pellicles. In four days the clouding in the 2, 4, and 10 drop tubes had disappeared, but two days later it returned, and a definite and continued growth followed.

While it still showed inhibition, one of the cultures was used for refiltering and exposure again to pH 7.0 beef-bouillon cultures of the hop strain, and 2, 10, 15, 20, 25, and 30 drops of filtrate were added to the fresh hop cultures. There was inhibition of growth in all for four days. Although the inhibition seemed to be a case of lysis and there was no trace of growth in the first refiltering with exposure to the organism, 1 and 2 drops of one of the inhibited cultures were added to slightly clouded fresh cultures of the hop strain. If the bacteriophage was potent, it was thought that it would clear up the clouding in these tubes. This did not occur, for there was a heavier growth in them in 24 hours. Later the inhibited culture itself became clouded.

The inhibition was more marked in the third passage than in the first and second, and freshly clouded cultures of hop strain were cleared by 10-drop and 1 c. c. additions of one of the inhibited cultures. The clearing, however, lasted for only three days, when clouding began.

The inhibition of cultures occurred likewise in the sixth and seventh passages for a few days, and during this time clouded cultures were

treated with them to see whether lysis would occur. In the sixth passage this clearing did not take place, but further growth was stopped for 3 days. In the seventh-passage tests there was a clearing in the two cultures under observation which lasted 1 and 2 days, but typical *Bacterium tumefaciens* growth began on the third and fourth days. The seventh-passage inhibited cultures were used against more clouded hop-strain cultures, and the clearing, followed later by clouding, was repeated. No lysis occurred throughout the series.

As the seventh-passage inhibited cultures were used up in testing their ability to produce lysis in the clouded cultures, a third experiment was started with a fresh lot of rotted-carrot juice which had been filtered several times. The pH of the juice was 9.6 after filtering.

Along with this series of passages of rotted-carrot filtrate and the organism, filtered juice of healthy normal carrot exposed to the hop strain of *Bacterium tumefaciens* was tested also. The passages with the two filtrates were carried with the hop strain only, and 1-day-old beef-bouillon cultures were used for seeding pH 6.7 beef-bouillon tubes to which the filtrates were added.

In the third passage of the rotted-carrot filtrate there was inhibition for 13 days and in the fourth passage for 20 days. The increase in inhibition did not continue, for it dropped to 6 days in the fifth passage, increased to 7 days in the sixth, and fell back to 6 days in the seventh, when the passages were discontinued.

The inhibition in the normal-carrot filtrate exposed to the hop strain was four days at the third passage but dropped back to two days in the fourth and fifth passages.

BACTERIOPHAGE PLAQUES

The bacteriophage plaques on beef-agar slants and plates were studied more extensively in the third experiment with the rotted-carrot filtrate. The plaques appeared on the normal-carrot filtrate plates and slants (fig. 2, A) exposed to the hop strain as well as on slants and plates poured from the rotted-carrot filtrate (fig. 3, A; fig. 4, A, C, D, E, F) exposed to the hop strain, although they were not so numerous or so well defined. In addition to these, definite bacteriophage plaques occurred on the control plates (fig. 2, B; fig. 3, B; fig. 4, B), a thing that had not occurred before in the work. Table 5 gives some of the details of the third experiment with the rotted-carrot filtrate.

The control plates and slants showed plaques only when they were made from cultures weakened by frequent transfers and grown in a medium not too favorable. Figure 4, C, illustrates an agar-slant control culture which showed no plaques, while the agar slants of the rotted-carrot filtrate, third passage with *Bacterium tumefaciens*, seeded in the same way (fig. 4, E and F) showed many.

FIGURE 2.—Bacteriophage plaques on beef-agar plates: A, Sixth passage of *Bacterium tumefaciens* (hop strain) with filtrate of normal carrot; B, control, showing plaques less numerous but more striking than in A

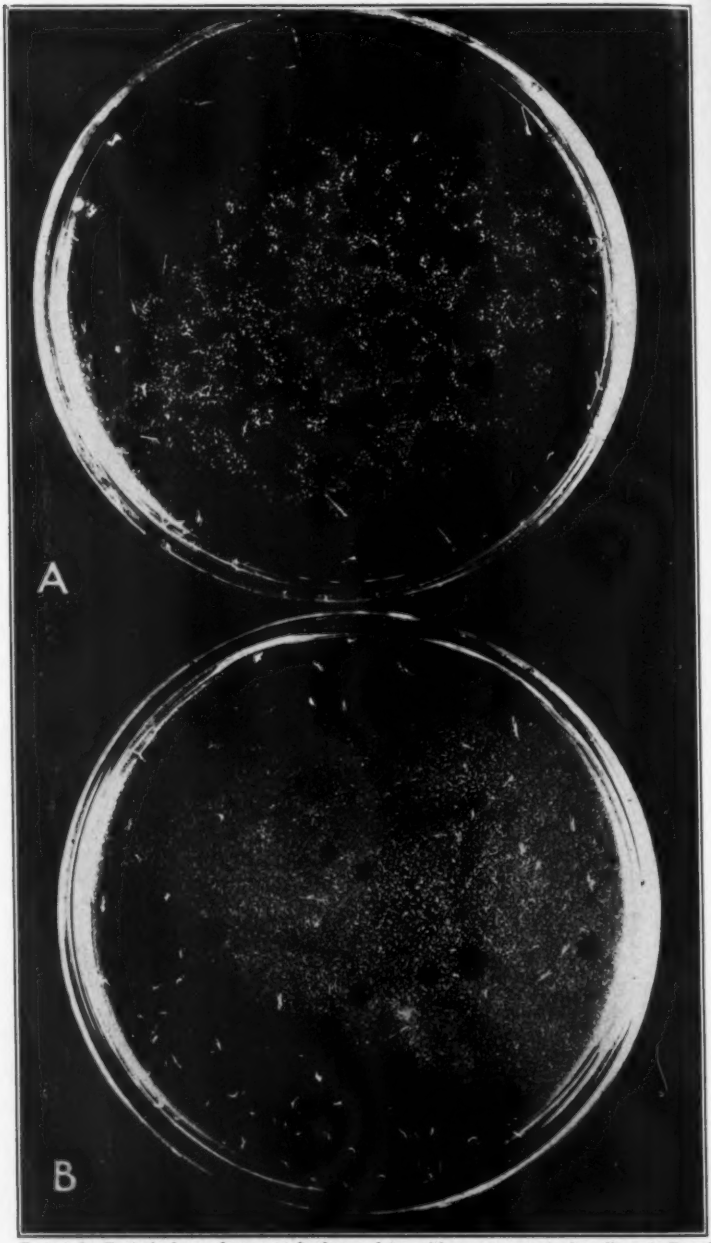

FIGURE 3.—Bacteriophage plaques on beef-agar plates with same amount of seeding: A, Fourth passage of *Bacterium tumefaciens* (hop strain) with filtrate of carrot rotted with *Bacillus carotovorus;* B, control plate of *Bact. tumefaciens* (hop strain), showing plaques also but not so numerous as in A

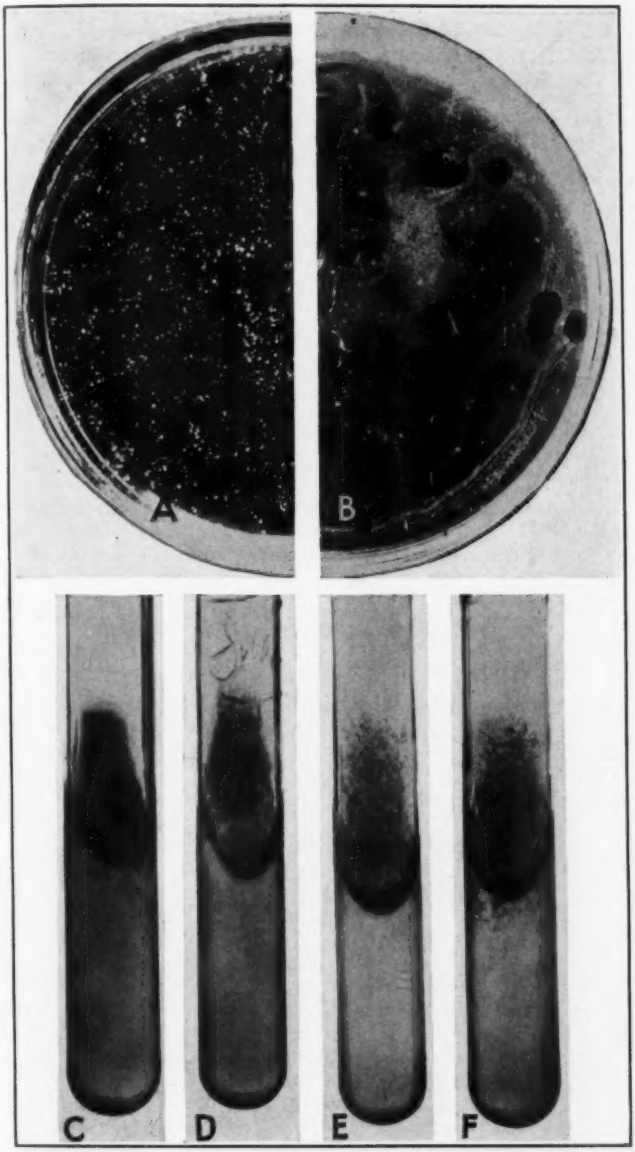

FIGURE 4.—Bacteriophage plaques of *Bacterium tumefaciens* (hop strain) on beef-agar plates and in tubes. A, Sixth passage of the organism with filtrate of rotted carrot. Note the presence of many plaques; B, Control. Note the presence of a few plaques; A and B received the same amount of seeding; C, Control culture seeded with two drops of a 24-hour beef-bouillon culture (plaques absent); D, Third passage of the organism with rotted-carrot filtrate swabbed on slant agar (tiny plaques present); E and F, Seeded with 2 drops of the third passage of the organism with the rotted-carrot filtrate (tiny plaques present)

TABLE 5.—*Effect of filtrates of rotted and normal carrots on Bacterium tumefaciens (hop strain) and the resulting bacteriophage plaques*

Passage	Filtrate of rotted carrot added to culture			Filtrate of normal carrot juice added to culture			Control culture in pH 6.7 beef bouillon	
	pH of filtrate before adding to culture, (pH 9.6 before passage with culture)	Inhibition (days)	Bacteriophage plaques	pH of filtrate before adding to culture (pH 6.8 before passage with culture)	Inhibition (days)	Bacteriophage plaques	Growth	Bacteriophage plaques
First	7.0	3	Few	7.1	1	Few	Good in 24 hours.	None.
Second	7.1	4	do	6.6	2	do	do	Do.
Third	6.8	13	More than with second passage.	6.7	4	do	do	Few.
Fourth	6.7	20	Abundant	6.7	2	Abundant	do	Moderate number; 25 on some plates.
Fifth	[a] 7.8	6	do	6.0	2	None	do	Few and inconspicuous.
Sixth	6.2	7	do	6.2	[b]	Many	[c]	12 to 15 large ones on plates.
Seventh	6.7	6	Many, but not classic type.		[b]		[c]	Some, but not classic type.

[a] Portion of filtrate stood open three days before reading was made.
[b] Notes not taken for four days; good growth then.
[c] Notes not taken for four days; heavy clouding then.

The plaques were tested in beef-bouillon cultures to see if they could produce lysis. Pieces of several of the fourth-passage plaques were cut out of the agar plates of the rotted and normal filtrates containing the organism and the control plates and were added to slightly clouded beef-bouillon cultures. The beef-bouillon did not clear, but there was no additional growth for two days, showing that the plaques had some inhibitive power. A definite clouding began on the third day or a little before that time in some cultures.

Sterile cover glasses were dropped on plaques on agar plates, removed, stained with carbol fuchsin, and studied under the microscope. The plaque picture was reproduced on the cover glass, for the tiny bacterial colonies outlining the plaques stained well. These colonies were made up mostly of swollen forms, very short rods appearing as coccus forms, and some normal-sized bacteria also, but the greater number of the normal ones were back from the margin of the plaque. A few bacteria in the center of the plaques appeared as tiny rods. These may have been drawn into the center by the removal of the cover glass from the top of agar plates, or they may have been there all the time. It is possible that they were bacteria inhibited but not destroyed by the bacteriophage. Among the swollen bacteria there were tiny particles which perhaps were the remains of swollen forms that had burst.

TESTS WITH INHIBITED CULTURES

Although the cultures of the fourth passage showed inhibition and it was thought that there was a condition of lysis, hanging drops from them were examined under the microscope 10 days after the cultures were made. Very few bacteria were located, but those found were typical rod-shaped forms of *Bacterium tumefaciens* without movement.

To study the phenomenon further, 10 drops of one of the inhibited cultures were added to slightly clouded beef-bouillon tubes. The clouding cleared somewhat in 1 day, but in 2 days definite *Bacterium tumefaciens* growth began and made slow but good development. Three days after these cultures were made the original fourth-passage cultures began to cloud, showing that there was no longer inhibition. The inhibition had lasted 20 days.

INOCULATIONS WITH INHIBITED CULTURES

The cultures inhibited 7, 13, and 20 days never developed typical *Bacterium tumefaciens* growth after growth began, but transfers from them to beef bouillon, pH 7.0, did. The clouding in the former was not heavy, no pellicle formed, and there was a precipitate of a less viscid consistency than usual at the bottom of the tube.

Ricinus plants were inoculated with the 7, 13, and 20 day inhibited cultures after growth took place in them, and also with control cultures of *Bacterium tumefaciens* made at the same time. These cultures of the sixth, third, and fourth passages, respectively, were the ones that demonstrated the bacteriophage plaques so well.

Power to infect was lost with the third passage, in which inhibition lasted 13 days, for no tumors formed (fig. 5 A), although the control cultures produced full-sized tumors (fig. 5, B).

Inoculations with the fourth passage, in which inhibition continued for 20 days, showed that some bacteria were still infectious, for tumors were produced. There was marked retardation in their development, however, for in 15 days 2 inoculations out of 5 did not show any developing outgrowth, the other three showed mere traces or swellings, while the 3 control culture tumors were 1.75 to 2 cm. in diameter. After nearly 3 months one inoculation was still negative; 2 of the tumors were only 2 cm. and 2 were 5 cm. in diameter (fig. 5, C), while the control tumors had reached their full size (5 cm.) in less than 2 months and before 3 months were necrosing. (Fig. 5, D.)

Inoculations with the sixth passage where there was inhibition for 7 days also showed a retardation of tumor formation and the presence of some infectious bacteria, as in the fourth passage. In one month two tumors of the sixth-passage inoculations were only 4 mm., while one was 12 mm., in diameter. The controls were 2 to 2.5 cm. in diameter. One inoculation was negative, and at the end of three months it continued negative. The smallest tumor was 12 mm., and the largest were 2 and 4 cm. (Fig. 5, E.) The control tumors at the same time were all 5 cm. in diameter. (Fig. 5, F.)

As the plants used for the 7, 13, and 20 day inhibited-culture inoculations were of the same age, inoculated about the same time and grown under the same conditions, the difference in the time of appearance of tumors produced by these cultures (or the nonappearance

of tumors) was striking, as was also the marked difference in the sizes of the tumors within a series, irrespective of the size of the controls. The bacteria inhibited for a time by the bacteriophage and later able

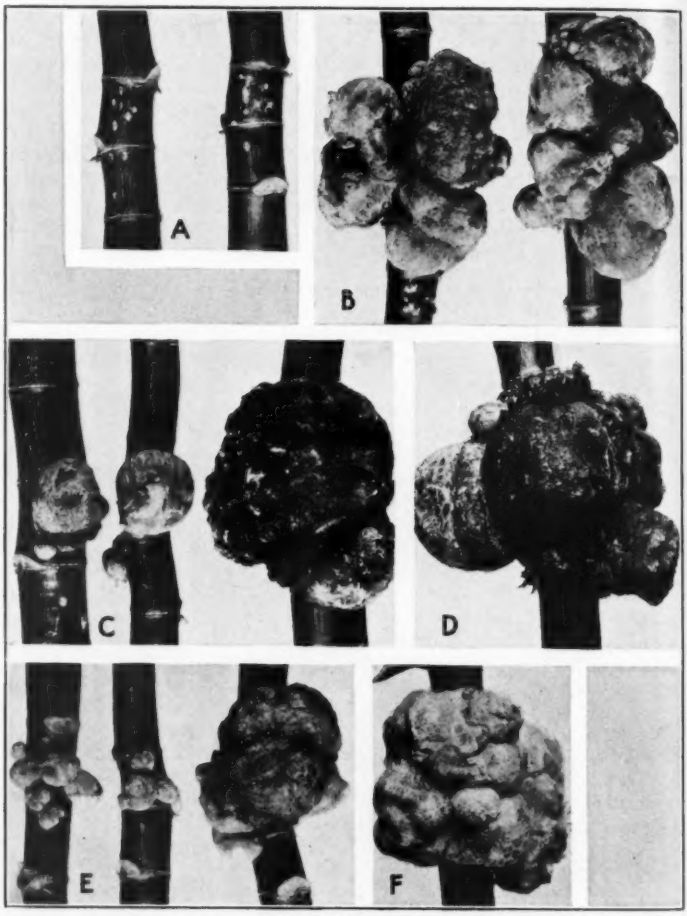

FIGURE 5.—Negative, retarded, and control infections produced by inoculating special cultures of *Bacterium tumefaciens* (hop strain) and control cultures of same organism into Ricinus plants. All reduced nearly one-half. A, Inoculation with third passage of *Bact. tumefaciens* and rotted-carrot filtrate. Photographed three months after inoculating; no infection. B, Control culture of *Bact. tumefaciens* inoculated into Ricinus plants. Photographed three months after inoculating. Regular-sized galls produced. Compare with A. C, Inoculations with fourth passage of the organism and rotted-carrot filtrate. Photographed more than three months after inoculating. Two tumors undersized and one nearly normal size. (One negative not shown.) D, Control culture inoculated into Ricinus and photographed at the same time as C. Tumor normal size. Compare with C. E, Inoculations with sixth passage of organism and rotted-carrot filtrate. Photographed three months after inoculating. Two tumors undersized and one nearly normal. (One negative not shown.) F, Control culture inoculated into Ricinus and photographed at the same time as E. Tumor normal size. Compare with E

to overcome its action do not seem to retain their infectious ability to the same degree. A young normal culture of the hop strain of *Bacterium tumefaciens* inoculated into Ricinus gave 100 per cent

infection, the tumors varying but little in size when conditions were similar. The size of mature tumors produced by a normal culture of hop strain on Ricinus depended on the age of the plants at the time of inoculation, the time of year inoculated, and the rapidity with which the plants were grown. The size at maturity may range from 1.75 to 6 cm. Table 6 shows the results of the inoculations with the third, fourth, and sixth passage inhibited cultures after growth had taken place in the cultures.

TABLE 6.—*Results of inoculating Ricinus plants with equal-aged inhibited and control cultures of Bacterium tumefaciens*

Description of culture	Time after inoculation	Inoculation with inhibited cultures			Inoculation with control cultures		
		Plants inoculated	Tumors produced	Diameter of of tumors	Plants inoculated	Tumors produced	Diameter of tumors
		Number	*Number*	*(Mm.)*	*Number*	*Number*	*Mm.*
Third passage of Bacterium tumefaciens with rotted carrot filtrate in which growth did not begin until after 13 days of inhibition.	1 month	4	0		3	3	20–25
	2 months	4	0		3	3	35–40
	3 months	4	0		3	3	45–50
Fourth passage in which growth was inhibited 20 days.	10 days	5	0		3	3	4–5
	15 days	5	3	2–3	3	3	17–20
	24 days	5	3	3, 4 and 12	3	3	40–45
	1 month, 17 days.	5	[a] 3	3, 20, and 25.	3	3	50
	2 months, 23 days.	5	4	20 and 50	3	3	[b]
	3 months, 15 days.	5	4	25 and 50	3	3	[c]
Sixth passage in which growth was inhibited 7 days.	1 week	4	0		3	0	
	1 month	4	3	4 and 12	3	3	20–25
	2 months, 4 days.	4	3	6, 12, and 25.	3	3	45–50
	3 months	4	3	12, 20, and 40.	3	3	50

[a] Also one starting to swell. [b] Beginning to necrose. [c] Badly necrosed.

REPETITION OF D'HÉRELLE AND PEYRE'S EXPERIMENT IN WHICH THEY PRODUCED TUMORS BY A FILTERING FORM OF BACTERIUM TUMEFACIENS

In 1927 D'Hérelle and Peyre (*4*) published a report of some of their work with plant tumors produced by *Bacterium tumefaciens*. They believe that there are two kinds of colonies isolated from the tumor, one an ultrapure colony, nonparasitic, and another contaminated by the bacteriophage, which is the infectious colony. They believe also that the infection of the bacteria by the bacteriophage produces invisible forms of the bacteria, which they call protobacterial forms; and with the filtrate from plant tumors they have been able to obtain the return of the filtering protobacterial form to the normal bacterial form. Inasmuch as the microscopic examination of tumors does not show any bacteria, they suggest that the true parasite is the protobacterial form of the organism. These protobacterial forms they believe can act as virus filtrates and when inoculated into susceptible plants produce tumors. Their experiments with a filtrate from sugar-beet tumors induced by inoculating with *Bacterium tumefaciens* bore out this belief. Out of 20 sugar beets inoculated with the filtrate, 14 produced tumors within 70 days, and platings from 6 of the tumors gave typical cultures of *Bacterium tumefaciens*. According to D'Hé-

relle's hypothesis, the relation of the bacteria parasitized by the bacteriophage is a symbiotic one. This symbiosis has become stronger through continued passages through living hosts, and it is very difficult to break up, sometimes impossible with the methods now known.

The writers carried out the same experiment using Ricinus tumors for filtering. As soon as the filtrate was obtained, it was inoculated into 40 Ricinus plants with a hypodermic needle. The plants were kept under observation for several months, but no tumor appeared. The experiment was made three times and with three different lots of tumors. Each time other Ricinus plants of the same age were inoculated with a culture of *Bacterium tumefaciens* and held as controls. These control cultures always produced sizable tumors.

Poured plates of the three filtrates were made and studied for sterility. No colonies of *Bacterium tumefaciens* appeared. However, nine other colonies of several types were observed on the three sets, and, in the belief that these might be of value should there be a protobacterial form, they were transferred, cultured, and inoculated into Ricinus plants. No tumor arose from these vagrant colonies. In all probability they were air colonies that had no connection with the filtrates, and the filtrates themselves were free from *Bacterium tumefaciens*, as the hypodermic inoculations showed.

In order to test further the presence of an infectious filtering form of *Bacterium tumefaciens*, flasks of beef bouillon, pH 7.0, were inoculated with the hop strain, left to grow for 3 days, and then filtered. Sixteen Ricinus plants were inoculated hypodermically with some of the filtrate, and other Ricinus plants of the same age were inoculated with a 3-day-old control culture of the hop strain. Plates were poured from some of the filtrate to test its sterility. The plates were examined during a period of 11 days, but no colonies of *Bact. tumefaciens* appeared. The three colonies that came up on the plates were transferred, and inoculations into Ricinus were made with subcultures. The inoculations with both the sterile filtrate and the three suspicious colonies were kept under observation more than 2 months, but there was no trace of outgrowths. The control culture inoculations produced large tumors.

Part of the sterile beef-bouillon filtrate was held in the laboratory to give an opportunity for the filtering bacterial form to develop if such form occurs. There was a clouding in the filtrate in four days, but it was not a typical *Bacterium tumefaciens* growth. However, inoculations into Ricinus plants were made with the clouded filtrate because of the possibility that the growth might be a filtering infectious form of *Bact. tumefaciens* as D'Hérelle and Peyre assert. These inoculations were watched for two and one-half months, but no trace of tumor growth occurred.

DISCUSSION

The conditions required for obtaining a culture of *Bacterium tumefaciens* that will produce more quickly growing and larger tumors than the controls can be brought about readily by adding a highly diluted tumor filtrate to a young culture of the organism and letting it grow two to four days before inoculating with it. There is some evidence

that filtrates of normal-plant juices also have the ability to produce more rapidly growing and larger tumors than the controls, although this evidence is based on experiments with normal Ricinus and normal carrot filtrates only.

A sewage filtrate combined with the organism produced faster growing and larger tumors than a filtrate of a sugar-beet tumor combined in the same way. The latter in turn produced more rapidly growing and larger tumors than the controls. A filtrate to which the organism was exposed as soon as possible after the tumor was crushed and filtered gave a culture which seemed to be nearly as effective in producing the rapid-growing and oversized tumors as a filtrate which had oxidized several days.

It may be that the addition of the fresh filtrate cements the union of the bacteria and the bacteriophage more closely and a higher degree of virulence is temporarily established.

No absolute lysis was produced in these experiments. It is quite possible, however, that the apple or rose strains of *Bacterium tumefaciens* might be cultured with some tumor filtrate and produce it. These two show evidence of being about the weakest strains of *Bact. tumefaciens*. This weakness, which manifests itself in producing tumors slowly when these strains are inoculated into susceptible plants, even into their native hosts, and the inability of these strains to produce tumors in some of the common plants easily grown in greenhouses, kept the writers from pursuing the development of lysis with the apple and rose strains through successive passages with a filtrate. The writers are assuming that the weakness of the strains is linked with the more facile separation of the bacteriophage from the organism. Very young cultures of the organism seemed to be necessary for the passages with the filtrates to produce inhibition. To obtain young cultures frequent transfers were required, which apparently had a tendency to bring about an instability of the close union that had become established between the bacteriophage and the organism and that allowed a release of the bacteriophage. In this temporary release the bacteriophage could affect the organism by its own power to cause inhibition. This release is manifested also by means of the bacteriophage plaques on agar slants and plates, and if a high potency has been reached, by marked inhibition. No doubt a condition could be brought about where there would be complete lysis. The culture that was inhibited for 13 days did not produce tumors when inoculated into Ricinus plants, although it was not used for inoculations until a few days after growth appeared. The control cultures of the same age produced tumors. An inhibited culture of 20 days produced tumors, retarded in appearing, but nevertheless present. The organisms resistant to the bacteriophage in the latter case were evidently of an infectious nature, while in the former they were not.

The bacteriophage plaques appearing on the plates poured from a pure culture of the hop strain of *Bacterium tumefaciens* as well as on those poured from filtrate *Bact. tumefaciens* cultures indicate that the lytic or inhibiting principle is carried along with the growth of the organisms irrespective of any active filtrate. The plaques from a pure culture were large and distinct but never so numerous as those on the filtrate-culture plates.

Sometimes there was a tumor-juice filtrate carried in passages with *Bacterium tumefaciens* which seemed not to activate the young culture, and if there was no inhibition of the organism in the early passages, it did not appear in a later passage. Whether the age of the tumor, the host of the tumor, or the rapid or slow rate at which the tumors had developed played a part in the presence and potency of the bacteriophage in the extracted juices was not fully determined. Frequently there was some evidence for thinking so. For instance, a sterile filtrate of old Ricinus tumors with necrosed areas gave no indication of inhibition when *Bact. tumefaciens* was exposed to it with three refilterings, nor at the beginning did it give evidence of producing acceleration of growth. The filtrates of very young tumors were not found satisfactory to work with. Whether this was because of the age or because the bacteriophage adhered to the more colloidal juice and did not pass through the filter is not known. It was found more difficult to filter the juice extracted from very young tumors. Those tumors not yet mature were found quite satisfactory and their filtrates could produce both inhibition and acceleration of growth when *Bact. tumefaciens* was exposed to them.

SUMMARY

The potentials of juices of normal and tumor tissue and *Bacterium tumefaciens* in culture revealed the following facts: (1) The pH of the freshly extracted juice of tumor tissue was always higher, that is, the reaction was more alkaline than that of the normal tissue; (2) the total acid content was greater in the tumor juice than in the normal juice; (3) the oxidation potential was always greater (more negative) in the freshly extracted tumor juice than in the normal juice—that is, the electrical charge carried by the gold electrodes in the fresh tumor juice was always negative (−) to that in the normal juice; (4) the electrical charges (accompanied always by a change in pH) on the gold electrodes were exactly reversed in the juices of the normal and tumor tissues upon further oxidation; (5) the change in pH produced by the growth of the organisms *Bact. tumefaciens* in beef-bouillon culture and *Bacillus carotovorus* inoculated into fresh carrots, progressed in the same direction (pH increase) as the change of pH of the normal juice upon further oxidation rather than in the direction (pH decrease) of the pH changes of the tumor juices; (6) the presence of a bacteriophage was established.

The presence or absence of plaques on poured plates appears to be correlated with the rise and fall of the pH of the plant filtrate or the pH of the culture of *Bacterium tumefaciens* treated with the filtrate. The pH value of the *Bact. tumefaciens* cultures which produced plaques when only 24 hours old was 6.7, and that of a normal *Bact. tumefaciens* culture 1 month old was 8.2. No plaques were present. A *Bact. tumefaciens* culture treated with a *Bacillus carotovorus* filtrate of pH 9.6 after 24 hours was pH 7.1 and after 4 days was pH 6.6, or slightly more acid than the fresh normal culture. This culture treated with *B. carotovorus* filtrate produced a greater number of plaques than *Bact. tumefaciens* cultures treated with other filtrates such as normal carrot, Ricinus tumor, or *Bact. tumefaciens*.

Accelerated growth of bacteria with increased pathogenicity seems to be associated with the phenomenon of bacteriophagy as well as does the retarded or inhibited growth of bacteria, causing either decreased or delayed pathogenicity or none at all.

The accelerated growth of the bacteria and increased size of tumors as compared with those of the controls were induced by the addition of high dilutions of tumor filtrates to young cultures used later for the inoculations. There is some evidence that filtrates of normal plant juices likewise have the ability to produce larger and more rapidly growing tumors than the controls. Inhibition of the bacteria from 4 to 20 days was obtained by successive passages of the tumor filtrate and light suspensions of the bacteria through Chamberland L3 filters. Tumors of small size or none at all were obtained through inoculations with these inhibited cultures after growth took place in them. Slight dilutions of the tumor filtrate grown with the culture inhibited the growth of bacteria and retarded tumor development. Tumors produced by these inhibited cultures occasionally reached the size of the control tumors, after a long slow growth. Complete lysis, the dissolving of all the bacteria in a culture, did not take place in the course of the work, which continued more than a year.

Bacteriophage plaques were obtained from the plant-tumor filtrates combined with *Bacterium tumefaciens*. The greatest number and largest ones, however, were obtained on beef-agar plates and slants from the rotted-carrot filtrate to which *Bact. tumefaciens* (hop strain) was exposed.

Typical bacteriophage plaques were obtained also on beef-agar plates and slants from a pure culture of *Bacterium tumefaciens*, and also in plates poured from a normal-carrot filtrate combined with *Bact. tumefaciens*. These plaques were not so numerous as those on the rotted-carrot filtrate plates.

D'Hérelle and Peyre assert that there exists a bacterial filtering form of *Bacterium tumefaciens* which is the infectious principle and which can be cultivated back to the nonfiltering form. Three repetitions of their experiment with sterile filtrates of *Bact. tumefaciens* tumors and also one with a sterile filtrate of beef-bouillon cultures of the organism were carried out, but the results were negative.

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NUTRITIVE VALUE OF PROTEINS IN CERTAIN KINDS OF SAUSAGE AND OTHER MEAT FOOD PRODUCTS[1]

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INTRODUCTION

Sausage and other meat food products manufactured in whole or in part from chopped meat, meat by-products, and condiments occupy an important place in the dietary of the American people. The estimated total production of sausage and sausage casings in the United States for the year 1925 was approximately 1,366,849,000 pounds, exclusive of sausage manufactured by small retail butchers and provision dealers and by farmers. These figures, after correction for exports and imports, indicate a per capita consumption of 11.6 pounds per annum.[2]

Sausage and certain other meat food products are prepared chiefly from two groups of ingredients, viz, meat and meat by-products. The term "meat"[3] denotes the flesh of cattle, swine, sheep, and goats. The meat used in the manufacture of sausage consists chiefly of trimmings from the carcasses of swine and cattle, but it may also include certain cuts and even entire carcasses of these animals. The term "meat by-product" denotes edible organs and parts, other than meat, obtained from the carcasses of cattle, swine, sheep, and goats. Characteristic condiments are used in the preparation of each kind of sausage. Cereal, usually corn flour, may be added to the extent of 3.5 per cent to sausage manufactured in federally inspected establishments provided the product is marked to indicate the presence of cereal. If products prepared in simulation of sausage contain substances foreign to sausage, or excessive quantities of cereal or moisture, they must be branded "Imitation."

In a general way, each kind of sausage or other meat food product is prepared from a certain group of ingredients, but the kinds and proportions used may vary considerably in different establishments. Some manufacturers prepare more than one grade of the same product, using a different formula for each grade. Naturally the best grade is made from the choicest materials.

There is much information in the literature concerning the chemical composition of sausage and other meat food products, but these data do not fully indicate the nutritive value of these products. Information concerning the vitamins in sausage and the biological value of sausage proteins is meager or lacking. The writers have already studied the nutritive value of the proteins in a number of kinds of

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[2] These figures have been calculated from data in reports of the Bureau of the Census and the Bureau of Foreign and Domestic Commerce, U. S. Department of Commerce, 1925.

[3] UNITED STATES DEPARTMENT OF AGRICULTURE, OFFICE OF THE SECRETARY. MEATS AND THE PRINCIPAL MEAT PRODUCTS. U. S. Dept. Agr. Food Insp. Decision 205, 3 p. 1926.

meat and meat by-products [4] used in the manufacture of sausage and have found considerable differences in the biological values of the proteins in certain of these products. It seemed likely, therefore, that material differences might be found in the nutritive values of the proteins in different kinds of sausage and other meat food products. The purpose of the experiments reported in this paper was to compare the nutritive values of the proteins in certain of the more common kinds of sausage and other meat food products.

DESCRIPTION OF MEAT FOOD PRODUCTS TESTED

The following meat food products were tested: Frankfurter-style sausage (two grades), Bologna-style sausage (two grades), pure pork sausage, fresh link sausage other than pure pork, Braunschweiger-style sausage, liver pudding or sausage, headcheese, scrapple, and meat loaf. For purposes of comparison, fresh pork ham and beef chuck were also tested. The above-named products were purchased direct from four meat-packing establishments operating under Federal inspection.

Frankfurter-style sausage of the best quality is prepared from meat, usually a mixture of beef and pork trimmings and certain beef cuts, and seasoning. The meat is ground very fine, mixed with salt and spices, and stuffed in animal or artificial casings. The sausage is then smoked, cooked for a short time in hot water, and finally chilled. Cheaper grades of Frankfurter-style sausage are made from a mixture of meat, meat by-products, and seasoning. Cereal is frequently added to these grades of sausage.

Bologna-style sausage is prepared from similar ingredients and in a like manner to Frankfurter-style sausage, except that the former is stuffed in larger casings or other containers and is cooked for a longer time. The cheaper grades of this product are made from much the same materials as those used for the same grades of Frankfurter-style sausage. Kosher Bologna-style sausage and kosher Frankfurter-style sausage contain no pork.

Pure pork sausage is prepared exclusively from pork and seasoning. The pork usually consists of lean-pork trimmings, but certain lean-pork cuts are used to a limited extent. The ground mixture is stuffed in animal casings or is packed in other containers.

Fresh link sausage, not pure pork, is made from beef and pork trimmings and seasoning, with or without the admixture of meat by-products. Cereal is frequently added.

Braunschweiger-style sausage is a high-grade liver sausage prepared from livers, pork trimmings, and seasoning. The product is thoroughly cooked and then stuffed in large hog casings.

Liver pudding or liver sausage is manufactured chiefly from meat by-products and may contain a moderate proportion of meat. Cereal is frequently added to this product. Liver pudding is thoroughly cooked in the process of manufacture.

[4] HOAGLAND, R., and SNIDER, G. G. NUTRITIVE VALUE OF THE PROTEIN IN VEAL AND CALF SWEETBREADS; IN BEEF CHEEK MEAT, LIPS, TONGUES, BRAINS, SPLEEN, AND TRIPE; AND IN HOG BRAINS AND TONGUES. Jour. Agr. Research 32: 679–688. 1926.

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Headcheese is usually prepared from a variety of meat by-products from swine and cattle, together with seasoning. This product may contain a moderate proportion of meat. Pig tongues are a frequent constituent of headcheese.

Scrapple is prepared chiefly from cereals, usually corn meal, buckwheat flour, or a mixture of the two with a variety of meat by-products, occasionally meat, and seasoning. The several ingredients are mixed with water, cooked to the desired consistence, and molded in pans. This product is sliced and fried before eating.

Meat loaf is usually prepared from a mixture of beef, pork, cereal, and seasoning. Occasionally veal is used. The finely ground mixture is molded in pans and thoroughly cooked. Loaves, other than true meat loaves, are prepared from a mixture of meat, meat by-products, and cereal.

METHODS

The procedure followed in these experiments was essentially the same as that previously used by the writers in a study of the nutritive value of the proteins in various animal tissues.[5] The proteins in the several kinds of sausage and other meat food products were compared by feeding tests with young male albino rats. Each ration was fed for 60 days to 6 rats weighing approximately 40 gm. each and not exceeding 30 days in age at the beginning of the test. The rats fed each ration were selected from three litters. The animals were weighed twice weekly and an accurate record was kept of the feed consumed by each.

Protein was fed at the 10 per cent level. Sufficient dried meat food product was used in each ration to furnish the desired proportion of protein and the rations were made adequate in other respects for normal growth. In addition to the meat food product, each ration contained the following constituents: Ash mixture, 4 per cent; cod-liver oil, 2 per cent; concentrated yeast extract, 2 per cent; hydrogenated cottonseed oil, 8 per cent; and sufficient cassava starch to make 100 per cent. The starch was practically free from nitrogen, but the yeast extract contained approximately 8 per cent nitrogen.

The meat food products were first separated from their containers, ground fine, and dried in a current of warm air. The dried products were ground, thoroughly extracted with ether, and analyzed for nitrogen.

RESULTS OF EXPERIMENTS

In Table 1 are presented the results of the first 30 days' feeding, and in Table 2 are given the data for the entire 60-day test. Only the average data for each group of six rats are recorded.

Ten per cent of meat protein of high biological value is not quite sufficient for normal growth in rats under the conditions of these experiments. Hence, material differences in the nutritive values of the proteins of the various meat food products were readily detected. Gain in weight without reference to protein intake is, of course, not an adequate basis for comparison.

[5] HOAGLAND, R., and SNIDER, G. C. THE VALUE OF BEEF PROTEINS AS A SUPPLEMENT TO THE PROTEINS IN CERTAIN VEGETABLE PRODUCTS. Jour. Agr. Research 34: 297–308. 1927.

TABLE 1.—*Nutritive value of protein in sausage and other meat food products, compared with that in beef and pork, when fed to young male albino rats for a period of 30 days at a protein level of 10 per cent*

Source of proteins [a]	Average age and weight of experimental animals			Total food intake		Intake per gram gain in weight		Gain in weight per gram consumed	
	Age at start	Weight at start	Gain in weight in 30 days	Entire ration	Protein	Entire ration	Protein	Entire ration	Protein
	Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
No. 1 grade Frankfurter-style sausage, establishment A	29	40	43	180	18.0	4.19	0.42	0.24	2.39
Do	28	40	44	188	18.8	4.27	.43	.23	2.34
No. 1 grade Frankfurter-style sausage, establishment B	24	42	85	302	30.2	3.55	.36	.28	2.81
No. 2 grade Frankfurter-style sausage, establishment C	27	40	60	234	23.4	3.90	.39	.26	2.56
No. 1 grade Bologna-style sausage, establishment A	24	39	58	248	24.8	4.28	.43	.23	2.34
No. 1 grade Bologna-style sausage, establishment B	23	41	74	268	26.8	3.62	.36	.28	2.76
No. 2 grade Bologna-style sausage, establishment C	25	44	66	245	24.5	3.71	.37	.27	2.69
Liver sausage, establishment A	26	43	49	230	23.0	4.69	.47	.21	2.13
Liver sausage, establishment B	26	42	64	285	28.5	4.45	.45	.22	2.25
Braunschweiger-style sausage, establishment A	26	43	68	211	21.1	3.10	.31	.32	3.22
Braunschweiger-style sausage, establishment B	25	45	100	308	30.8	3.08	.31	.32	3.25
Pork sausage, establishment A	28	40	76	223	22.3	2.93	.29	.34	3.41
Pork sausage, establishment B	26	41	70	227	22.7	3.24	.32	.31	3.08
Fresh link sausage, establishment D	23	39	41	186	18.6	4.54	.45	.22	2.20
Beef chuck (cooked), establishment A	26	41	95	324	32.4	3.41	.34	.29	2.93
Beef chuck (uncooked), establishment A	27	41	101	329	32.9	3.26	.33	.31	3.07
Pork (fresh ham), establishment B	25	40	125	340	34.0	2.72	.27	.37	3.68
Do	26	41	102	307	30.7	3.01	.30	.33	3.32
Meat loaf, establishment A	25	41	62	199	19.9	3.21	.32	.31	3.12
Meat loaf, establishment B	25	39	107	308	30.8	2.88	.29	.35	3.47
Scrapple, establishment A	27	44	34	172	17.2	5.06	.51	.20	1.98
Scrapple, establishment E	27	42	74	258	25.8	3.49	.35	.29	2.87
Headcheese, establishment A	27	41	26	165	16.5	6.35	.63	.16	1.58
Headcheese, establishment B	26	41	34	175	17.5	5.15	.51	.19	1.94

[a] Each lot of sausage or other meat food product was fed to six rats; results given are averages.

From the data presented in Table 1, the meat food products may be divided into three groups based on the apparent nutritive values of the proteins in those products. In the first group are included products containing proteins which showed the highest nutritive values, viz, pork sausage, Braunschweiger-style sausage, and meat loaf. In the second group are included products containing proteins which exhibited intermediate values, viz, Frankfurter-style sausage and Bologna-style sausage. Scrapple from establishment E also belongs in this group. In the third group are included those products containing proteins which seemed to be of lower nutritive value than those of the first two groups, viz, liver sausage, headcheese, and fresh link sausage. Scrapple from establishment A is included in this group.

For the first group, the gain in weight for each gram of protein consumed ranges from 3.08 gm. for pork sausage, establishment B, to 3.47 gm. for meat loaf, establishment B. The values obtained for the products in this group compare favorably with those recorded for beef chuck and fresh ham, viz, 2.93 to 3.68 gm.

For the second group the gain in weight per gram of protein consumed ranges from 2.34 gm. for Frankfurter-style sausage, establishment A, to 2.87 gm. for scrapple, establishment E.

For the third group the gain in weight for each gram of protein consumed ranges from 1.58 gm. for headcheese, establishment A, to 2.25 gm. for liver sausage, establishment B.

TABLE 2.—*Nutritive value of protein in sausage and other meat food products, compared with that in beef and pork, when fed to young male albino rats for a period of 60 days at a protein level of 10 per cent*

Source of proteins [a]	Average age and weight of experimental animals			Total food intake		Intake per gram gain in weight		Gain in weight per gram consumed	
	Age at start	Weight at start	Gain in weight in 60 days	Entire ration	Protein	Entire ration	Protein	Entire ration	Protein
	Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
No. 1 grade Frankfurter-style sausage, establishment A	29	40	96	468	46.8	4.88	0.49	0.21	2.05
Do	28	40	95	470	47.0	4.95	.49	.20	2.02
No. 1 grade Frankfurter-style sausage, establishment B	24	42	171	697	69.7	4.08	.41	.25	2.45
No. 2 grade Frankfurter-style sausage, establishment C	27	40	114	502	50.2	4.40	.44	.23	2.27
No. 1 grade Bologna-style sausage, establishment A	24	39	117	596	59.6	5.09	.51	.20	1.96
No. 1 grade Bologna-style sausage, establishment B	23	41	152	616	61.6	4.05	.41	.25	2.47
No. 2 grade Bologna-style sausage, establishment C	25	44	130	565	56.5	4.35	.43	.23	2.30
Liver sausage, establishment A	26	43	87	512	51.2	5.89	.59	.17	1.70
Liver sausage, establishment B	26	42	122	655	65.5	5.37	.54	.19	1.86
Braunschweiger-style sausage, establishment A	26	43	141	578	57.8	4.10	.41	.24	2.44
Braunschweiger-style sausage, establishment B	25	45	175	725	72.5	4.14	.41	.24	2.41
Pork sausage, establishment A	28	40	152	556	55.6	3.66	.37	.27	2.73
Pork sausage, establishment B	26	41	156	588	58.8	3.77	.38	.27	2.65
Fresh link sausage, establishment D	23	39	90	462	46.2	5.13	.51	.19	1.95
Beef chuck (cooked), establishment A	26	41	160	689	68.9	4.31	.43	.23	2.32
Beef chuck (uncooked), establishment A	27	41	166	693	69.3	4.17	.42	.24	2.40
Pork (fresh ham), establishment B	25	40	201	731	73.1	3.64	.36	.27	2.75
Do	26	41	193	727	72.7	3.77	.38	.27	2.65
Meat loaf, establishment A	25	41	135	523	52.3	3.87	.39	.26	2.58
Meat loaf, establishment B	25	39	186	716	71.6	3.85	.38	.26	2.60
Scrapple, establishment A	27	44	77	435	43.5	5.65	.56	.18	1.77
Scrapple, establishment E	27	42	137	543	54.3	3.96	.40	.25	2.52
Headcheese, establishment A	27	41	60	398	39.8	6.63	.66	.15	1.51
Headcheese, establishment B	26	41	67	402	40.2	6.0	.60	.17	1.67

[a] Each lot of sausage or other meat food product was fed to 6 rats; results given are averages.

If the meat food products are again classified according to the relative nutritive values of their proteins, based on the data for the 60-day experiments, the arrangement is slightly different, as follows: Group 1 includes pork sausage, Braunschweiger-style sausage, meat loaf, Frankfurter-style sausage, and Bologna-style sausage from establishment B, and scrapple from establishment E. Group 2 includes Frankfurter-style and Bologna-style sausage from establishments A and C. Group 3 embraces headcheese, fresh link sausage, and liver sausage. Scrapple from establishment A is included in this group.

For the first group the gain in weight for each gram of protein consumed during the 60-day test ranges from 2.41 gm. for Braunschweiger-style sausage, establishment B, to 2.73 gm. for pork sausage, establishment A. The range of values for this group compares favorably with that for beef chuck and pork hams, viz, 2.32 and 2.75 gm.

For the second group the gains for the 60-day period range from 1.96 gm. for Bologna-style sausage, establishment A, to 2.30 gm. for the same kind of sausage from establishment C.

For the third group, the gain in weight for each gram of protein consumed ranges from 1.51 gm. for headcheese, establishment A, to 1.95 gm. for fresh link sausage, establishment D.

DISCUSSION OF RESULTS

The results of the experiments reported in this paper indicate material differences in the apparent nutritive values of the proteins in the several meat food products examined. Based on these results, the products have been classified in three groups, but this arrangement applies only to the samples tested and does not have general application.

Attention is called to the fact that, among products from the same establishment, the proteins in Frankfurter-style sausage and in Bologna-style sausage had practically the same apparent nutritive value. These results were to have been expected because each establishment ordinarily makes the two kinds of sausage from essentially the same mixture of meats. It may be noted, however, that there are material differences in the nutritive values of the proteins in different lots of these two sausages purchased from different establishments.

The values obtained for the proteins in cooked and uncooked beef chuck, both samples being from adjacent parts of the same quarter of beef, agreed very closely for both the 30 and 60 day tests. The results obtained for two lots of fresh ham purchased at different times from the same establishment did not agree very well for the 30-day test but were very similar for the 60-day test. On the whole, the results indicate that the procedure followed in these experiments has given similar results with like products.

Considerable difference was found between the nutritive values of the proteins in the two lots of scrapple. During the 60-day test the rats getting scrapple from establishment A gained only 1.77 gm. in weight for each gram of protein consumed, whereas those fed scrapple from establishment E gained 2.52 gm. This difference is probably due to the use of a larger proportion of lean meat in the manufacture of scrapple at establishment E. It is noteworthy that, for the 60-day test, the protein in scrapple from establishment E had as high nutritive value as that in beef chuck. This fact is doubtless due to the supplemental relationship between the proteins of meat and those of corn meal, since both of these products were used in the manufacture of this lot of scrapple. The writers have previously reported concerning the value of beef protein as a supplement to that in corn meal.[6]

[6] HOAGLAND, R., and SNIDER, G. G. Op. cit. (See footnote 5.)

Attention has been called to the fact that, although each kind of sausage or other meat food product is usually made from a certain group of ingredients, there is nevertheless considerable variation in the practice at different meat-packing establishments. Not only may there be variations in the kinds and proportions of the different meats and meat by-products used, but there may also be differences in the quality of the ingredients as well. It is thus obvious that considerable variation may occur in the nutritive values of the proteins in the same type of sausage manufactured at different establishments. Hence the results of the experiments reported in this paper can not be taken as a basis for generalizations concerning the nutritive values of the proteins in the various products examined.

SUMMARY

The nutritive values of the proteins in certain kinds of sausage and other meat food products have been compared with those of beef chuck and fresh pork ham by feeding experiments with young albino rats. The results of these tests show that the proteins in certain products were of considerably higher nutritive value than the proteins in other products. The nutritive values of the proteins in samples of the same product manufactured by different establishments also varied.

Based on similarity in the nutritive values of their proteins, the meat products examined may be arranged in three groups. Group 1 includes those products containing proteins of the highest nutritive value, viz, all samples of pork sausage, Braunschweiger-style sausage, and meat loaf. The proteins in these products had practically the same nutritive value as those in beef chuck and fresh pork ham. Group 2 comprises products with proteins of lower nutritive value than those in Group 1, as follows: All samples of Frankfurter-style and Bologna-style sausage and one lot of scrapple from establishment E. Group 3 includes products with proteins of lower nutritive value than those in Group 2, viz, all samples of headcheese, liver sausage, and fresh link sausage, and one lot of scrapple from establishment A.

ARTIFICIAL SOILING OF COTTON FABRICS PREPARATORY TO LAUNDERING STUDIES[1]

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INTRODUCTION

Any scientific investigation dealing with laundering requires cloth soiled in a definite manner with known constituents. The study here reported was undertaken in order to develop a soiling mixture for use in experimental work on laundering being done in this laboratory. In this connection it was also necessary to study methods of applying the mixture to the fabric and of determining the amount of soil on the cloth after soiling and after the various laundering processes. Naturally soiled fabrics are not satisfactory because the nature and the quantity of the soil are not known, the soil is not distributed in a uniform screen, and the depth of the soil is not great enough to permit a gradation of grayness with variations in the washing process.

DISCUSSION OF FORMULAS AND METHODS USED BY OTHER INVESTIGATORS

Excluding stains, which have to be treated aside from the regular laundry process, there are four kinds of dirt commonly found on soiled clothing and household textiles: Albuminous material, such as eggs, blood, and body excretions; finely divided matter, such as soot and dust; saponifiable oils, including those of both animal and vegetable origin; and nonsaponifiable oils, such as mineral oils. Artificial soiling mixtures used by other experimental workers have contained substances that belong to one or more of these classes of compounds. Table 1 incorporates such formulas as have been found in the literature and obtained by private communication.

The choice of the component of the soiling mixture that gives the gray color to the test cloth has been the most troublesome problem. It must be chemically inert and insoluble in the detergents used, for any whitening should be an indication of the removal of soil by the washing process. Lampblack, carbon black, linden charcoal, benzol soot, and graphite have been used by the various investigators. (Table 1.) Elledge and Isherwood (*12*),[2] consider lampblack a substance comparable to soil in clothes since it consists of finely divided particles of carbon with various hydrocarbons condensed on them. However, it is rather unreliable in that each new supply may exhibit different characteristics. The American Oil Chemists' Society tried a suspension of lampblack in carbon tetrachloride as noted in formula

[1] Received for publication Mar. 23, 1929; issued October, 1929.

[2] Reference is made by number, (italic), to "Literature cited," p. 549.

5A (Table 1) but found it unsatisfactory. Formula 5C is now being used.

The use of carbon black, linden charcoal, and benzol soot may have been attempts on the part of the different investigators to employ a substance more constant in composition than lampblack. By microscopical examination, Butcher (*7*) finds that particles of lampblack (0.3–0.4 micron) are noticeably larger than those of carbon black (0.1–0.15). There is also a distinct difference between the two in that the latter mixes far more readily with water. Carbon black would therefore be favored above lampblack because its particles are smaller and it is more easily dispersed in water. A very useful form was found in news ink, which is a mixture of 10 per cent carbon black and 90 per cent mineral oil.

Graphite has been used in preference to lampblack in formulas 3, 12 and 16 (Table 1), and Chapin (*8, p. 461*) states that it has the advantage over any other form of carbon for detergency tests since it is free from porosity, and equilibrium in adsorption is reached in far briefer time. The British Launderers' Research Association, according to Parker (*28*), suggested the use of commercial preparations that eliminate the difficulty of wetting lampblack or finely ground dry carbon. One of these is available on the market as a suspension in water known as Aquadag, and another as a suspension in oil called Oildag. According to the manufacturer, they contain approximately 20 per cent and 12.5 per cent, respectively, of deflocculated graphite. These figures are higher than those reported by Parker (*28*). The extremely fine subdivision of the deflocculated graphite, which is in colloidal form, also makes it valuable for use in a soiling mixture. Aquadag is not so useful as Oildag for this purpose because of the greater difficulty in removing it from the fabric. Furthermore, its application is time-consuming, since a second dip in an organic solution of the oils is necessary after it has dried on the cloth.

Heermann (*14*) suggested the use of indigo paste instead of any form of dry carbon because this paste is always available in the same chemical composition and in the same mechanical form. Hirose (*18*) also reports the use of indigo as a component of his soiling mixture. However, if photometric readings are made to determine the amount of dirt removed by the washing process, blues are not suitable for comparison with a white standard. A black dye might be substituted for the indigo paste, but the question arises as to the difference in the power of hot and cold water to break down the mechanical attachment of the dye and the fiber. The same objection as to color can be raised against the reddish-brown burnt umber which was used in formula 9. (Table 1.)

There has also been a difference of opinion among investigators as to the most desirable oil or fat to include in the soiling mixture. Lindner (*23, 24*) believes that detergents that are good oil washers remove plant oils, animal fats, petroleum, resin, and, in fact, any organic substances of liquid or semiliquid nature just as well as mineral oil. He differentiates only between their "soot washing power" and their "oil washing power." In addition, present theories (Hillyer, *15, 16, 17;* Spring, *33*) are that soap cleanses by virtue of the undecomposed soap through emulsification, absorption, and the lowering of the interfacial surface tension at the oil-water interface, not because

TABLE 1.—*A review of various methods of applying different artificial soiling mixtures to te*

Investigator and literature citation number		Albuminous matter		Inert matter		Animal saponifiable oil		Vegetable saponifiable oil		Unsaponifiable oil	
		Kind	Quantity	Kind	Quantity	Kind	Quantity	Kind	Quantity	Kind	Quantity
Bergell (4)	(1A)	None		Linden charcoal.[a]	Not reported.	None		Soybean oil.[a]	Not reported.	None	
	(1B)	Do		do.[a]	do	do		None		Mineral oil[a]	Not reported.
Brauer (5)	(2A)	Do		None		do		Linseed oil	Not reported.	None	
	(2B)	Do		do		do		Rapeseed oil	do	do	
	(2C)	Do		do		do		Olive oil	do	do	
	(2D)	Do		do		do		Peanut oil	do	do	
	(2E)	Do		do		do		None		Paraffin oil	Not reported.
	(2F)	Do		do		do		do		Machine oil	do
	(2G)	Do		do		Margarine	Not reported.	do		None	
	(2H)	Do		do		Milk	do	do		do	
	(2I)	Do		Lampblack	Not reported.	None		Peanut oil	Not reported.	do	
	(2J)	Do		do	do	do		Linseed oil	do	do	
British Launderers' Research Association (Parker (28, p. 253)).	(3A)	Do		Aquadag	0.0068 gram	do		None		do	
	(3B)	Do		Oildag	0.04 gram	do		do		do	
	(3C)	Do		do	0.04 gram	do		Olive oil	0.04 gram	do	
Chapin (9, 10)	(4A)	Do		Carbon black	0.3 gram	do		None		do	
	(4B)	Do		Lampblack	10 grams	Lard	50 grams	do		do	
	(4C)	Do		do	10 grams	None		do		Vaseline	50 grams
	(4D)	Do		do	15 grams	do		do		Mineral oil	50 grams
American Oil Chemists' Society (2, 3, 13, 19, 20, 21).	(5A)	Do		do	2-3 grams	Tallow	3 grams	do		Lubricating oil	5 grams
	(5B)[b]	Do		do.[c]	20 grams	do.[c]	50 grams	do		do.[c]	30 grams
	(5C)[d]	Do		Lampblack Gum tragacanth Wheat starch	30 grams 6 grams 100 grams	do	20 grams	do		Mineral oil	20 grams
Elledge (11, p. 87)	6	Beef extract Egg albumin (dried)	1.5 grams 1 gram	Lampblack	2 grams	None		do		None	
Heermann (14):											
Indigo paste soil	(7A)	None		Indigo paste (20 per cent).	5 grams	do		do		do	
Fat-containing soil	(7B)	Do		do	5 grams	do		do		Mineral oil	50 grams
A size-containing, fat-free soil	(7C)	Do		Indigo paste (20 per cent). Starch	5 grams 10 grams	do		do		None	
Size and fat containing indigo paste soil	(7D)	Do		Indigo paste (20 per cent). Starch	5 grams 10 grams	do		do		Mineral oil	50 grams
Hirose (18)	8	Do		Indigo	1 gram	Lanolin	2 grams	do		None	
La Wall and others (1)	9	Do		Burnt umber	Not reported.	None		do		do	
Lindner (23, 24)	(10A)	Do		Benzol soot	do	do		do		do	
	(10B)	Do		None		do		do		Machine oil	Not reported.
Schiewe and Stiepel (30)	(11A)	Do		Linden charcoal	5 grams	do		do		Mineral oil	5 grams
	(11B)	Do		do	5 grams	do		do		(Fatty acid (60 per cent) Mineral oil (40 per cent)	do
	(11C)	Do		do	Not reported.	do		do		None	
Shorter (31, p. 109)	12	Do		Graphite	do	do		Olive oil	Not reported.	do	
Shukoff and Schestakoff (32)	13	Do		Lampblack	do	Lanolin	Not reported.	None		do	
The Hoover Co., private communication	14	Do		Lampblack[f]	13 grams	None		do		Mineral oil[f]	50 grams
Lever Bros. Co., private communication	15	Do		Carbon black	2 grams	Tallow	10 grams	do		Cylinder oil	17.5 grams
The Procter & Gamble Co., private communication.	16	Do		Carbon and graphite.	Not reported.	None		Linseed oil	Not reported.	None	
Waldrich Bleacheries, private communication.	17	Do		Lampblack	0.1 gram	do		Castor oil[e]	1 gram	Vaseline[e]	1 gram

[a] Equal parts.
[b] Superseded formula 5A.
[c] 10 gm. of paste made by grinding these ingredients in a paint mill.
[d] Superseded formulas 5B.
[e] Implied. Ackn
[f] One teaspoon of
[g] Used interchang

ures to textile fabrics used in the determination of washing efficiency

antity	Suspension medium: Substance	Suspension medium: Quantity	Test cloth	Method of applying soiling mixture to test cloth	Method of determining washing efficiency
	Ether	Not reported	Woolen cloth, 100 centimeters square.	Saturated with soiling mixture	Weight difference.
report-	do	do	do	do	Do.
	None		Cloth, 20 centimeters square.	Soaked in weighed quantity of soiling mixture	Ether extraction of washed samples.
	do		do	do	Do.
	do		do	do	Do.
	do		do	do	Do.
report-	do		do	do	Do.
do	do		do	do	Do.
	do		do	do	Do.
	do		do	do	Do.
	do		do	Dropped from measuring glass in equal quantities	Brightness comparison of washed samples with one as arbitrary standard.
	do		do	do	Do
	Water	17 cubic centimeters.	Cotton cloth, 10 by 14.5 inches, (9 grams).	Drawn through soiling mixture on horizontal glass plate, dried on frame, and resoiled.	Brightness comparison with color standards (color disks), and weight difference.
	Benzene	20 cubic centimeters.	do	Drawn underneath glass rod placed in tray containing soiling mixture and repeated once.	Do.
	do	do	do	do	Do.
	Water	30 cubic centimeters.	Nainsook, handkerchief linen, cotton shirting	Weighted strips agitated mechanically in test tubes for 8 minutes, and dried on glass plate at 100° C.	Brightness comparison with standard solution of cobalt, nickel, and copper salts in Kober-Klett colorimeter.
	None		Cotton poplin	Both sides smeared, excess scraped off, and dried on glass plate at 100° C.	Do.
rams	do		do	do	Do.
rams	do		do	do	Do.
ams	Carbon tetrachloride.	2,000 cubic centimeters.	Plain woven bleached cotton cloth.	Drawn through soiling mixture	Brightness comparison with color standards (Munsell gray paper scale, India-ink cloth color scale, standard gray pastes).
rams	do	do	do	do	Do.
rams	Water	824 cubic centimeters.	do	Printed in stripes	Number of washes to remove soil.
	do	4,000 cubic centimeters.	Handkerchiefs	Not reported	Brightness comparison of washed samples with one as arbitrary standard.
	do	1,000 cubic centimeters.	Desized cotton or linen cloth.	Dipped in soiling mixture until doubled in weight, and dried.	Brightness comparison of washed samples with one as arbitrary standard (brightness comparison with color standards suggested).
rams	Water Benzene or ether	do do	do	Dipped in inert matter and water and dried; then in oil solution and dried.	Do.
	Water	do	do	As in 7A	Do.
rams	Water Benzene or ether	do	do	As in 7B	Do.
	Alcohol and water. Petroleum ether	100 grams do	Muslin	Dipped in indigo-alcohol-water mixture, dried, and repeated, then dipped in fat solution and dried.	Brightness comparison with naked eye.
	Not reported		5-inch cloths	Equally impregnated with soiling mixture	Brightness comparison of washed samples with one as arbitrary standard.
	Alcohol	Not reported	Linen, 2.5 grams	Saturated with soiling mixture	Brightness comparison with color standards (Ostwald's gray scale).
t report- d.	None		do	Soaked in oil and excess squeezed out	Weight difference.
rams	Benzene	100 grams	Linen cloth	Applied with brush	Do.
do	do	do	do	do	Do.
	Water	Not reported	do	do	Do.
	None		Yarn	Treated yarn with soiling mixture to get even screen.	Not reported.
	Benzene	Not reported	Cotton cloth	Not reported	Brightness comparison of washed samples with one as arbitrary standard.
grams	Carbon tetrachloride.	4,543 cubic centimeters (1 gallon).	Muslin, 6 inches square.	Suspended in soiling mixture for 30 seconds and dried.	Brightness difference between soiled and washed samples by photometric readings.
5 grams	do	1,000 cubic centimeters.	Desized white cotton voile, 4.5 by 12 inches.	Shaken for 3 minutes in soiling mixture in closed jar and dried on brown paper.	Not reported.
	Turpentine	Not reported	Bleached cotton cloth	Passed through soiling mixture	Do.
ram	Carbon tetrachloride.	100 cubic centimeters.	Not reported	Shaken with soiling mixture and 100 grams glass beads.	Do.

ed. Acknowledgment is made of the translation of the article by Saburo Katsura, of the U. S. Department of Agriculture.
esspoon of this ground mixture.
interchangeably.

of the saponifying power (McBain, *25*, *26*, *27*) of the small quantity of alkali set free by the hydrolysis of even a neutral soap in aqueous solution. It is therefore probably unnecessary to include more than one oil to demonstrate the oil-removing power of the detergent employed, but an artificial soiling mixture which will simulate natural soil should perhaps contain a saponifiable animal oil, a saponifiable vegetable oil, and an unsaponifiable oil.

In choosing a representative of saponifiable oils, the standard groups—drying, nondrying, and semidrying—might be utilized, but this apparently has not been done in the formulas cited in Table 1. Of the animal fats used, tallow seems to be as satisfactory as any and is readily obtainable. High-quality olive oil is the least oxidizable of all vegetable oils, and is therefore an ideal representative of this group for a soiling mixture. Lanolin, though used in two of these formulas, is an animal wax rather than a fat or an oil. It would be useful as a component of a soiling mixture for wool-scouring investigations rather than for laundering experiments, since it is rarely a part of natural soil.

The only factor governing the selection of different mineral oils when they are included in the soiling mixture seems to be the color. A colored oil may introduce a yellow tone into the gray of the carbon and so make photometric readings more difficult. Refined oils are preferable since they may be better standardized.

The components of the dirt mixture should be dispersed in an oil-dissolving organic liquid, noninflammable, and reasonably volatile, with its boiling point somewhat below the boiling point of water but far above room temperature. Such a solvent permits the application of the inert and oily substances in one process and dries rapidly enough to avoid streaking of the soiled samples. Carbon tetrachloride is the only desirable one reported.

A study of Table 1 reveals a range of 0.04 to 0.2 gm. of carbon per 100 c. c. of medium, furnishing, doubtless, a great variety of soiled test cloths. However, the depth of soil obtained is due not only to the differences in the amount of carbon but also to the kind of carbon and the technic of soiling. If the 25 per cent brightness, corresponding to shade S48 of the Munsell gray scale, as earlier suggested by the committee of the American Oil Chemists' Society (*20*) is accepted as satisfactory, the amount of carbon and the length of time of soiling can be determined by trial.

The quantity of saponifiable and unsaponifiable oils included in the soiling mixture seems to have been an arbitrary matter with each investigator. In six of the formulas cited the total oily constituents range from 0.2 to 5.0 gm. per 100 c. c. of medium. The maximums are exhibited by the formulas of Brauer (*5*), Chapin (*10*), Lindner (*23*, *24*), and Shorter (*31*), who report the impregnation of the fabric with oil undiluted by any solvent. The important feature seems to be simply to have enough oil present to attach the inert matter to the fabric and thus to simulate natural soil. Equal quantities of saponifiable and nonsaponifiable oils would seem a fair test, the former being composed of equal weights of animal and vegetable oily matter.

A satisfactory method of applying soil to a test cloth deposits it in a uniform manner over the entire surface and permits reproduction in kind and depth of soil when another sample of the same cloth is

subjected to the treatment. The methods employed by different investigators comprise (1) agitation and shaking of the test cloth in the dirt mixture; (2) spreading the soil over the surface of the cloth; (3) passing the cloth through the soiling mixture; (4) printing in stripes; and (5) soaking the test cloth in the dirt mixture. Test cloths of a great variety of kinds and dimensions were used. Size is an important factor, for small samples may be soiled satisfactorily by a method that would not be at all adapted for larger samples of test cloth.

Few comments have been made by different investigators on the character of soiling achieved by a particular method employed. In the report of the work of the British Launderers' Research Association, Parker (*28*) states definitely that the method of soiling with Aquadag gives an even coating of graphite on the cloth. The color corresponds to "Grey II" and contains 0.0025 gm. of graphite per square foot. Grey II is one of the colors of the set of standard color disks made and used by the British Launderers' Research Association for measuring the tints of white goods in grading the work of power laundries. Soiling with Oildag, or with Oildag and olive oil, furnishes samples of color Grey II containing 0.08 gm. of Oildag or 0.08 gm. of Oildag with 0.08 gm. of olive oil per square foot after two treatments as described in Table 1 under formulas 3B and 3C, respectively. Heermann (*14*) admits that he did not succeed in producing uniform samples of even distribution of soil.

EXPERIMENTAL PROCEDURE AND CONCLUSIONS

STUDY OF THE DARK COMPONENT FOR THE SOILING MIXTURE

In developing a soiling mixture for use in this laboratory, tallow, olive oil, and a refined mineral oil were chosen as representative of the necessary fats and oils. Carbon tetrachloride was used as the medium when possible on account of its fat-dissolving power as well as its noninflammability. Since there is such a wide difference of opinion concerning the best dark constituent for such mixtures, this matter was given detailed study. Aquadag, Oildag, news ink, lampblack, Cibanone black B, and benzol soot were each considered with reference to uniform distribution after soiling and washing, the ease of removal of soil by the washing process, and the possibility of duplicating the brightness after soiling and washing by use of the same procedure.

In order to select the cotton fabric suitable for this work various kinds of plain woven cloth of different thread count and weight were soiled with an Oildag mixture and washed according to the procedure described (p. 546). A sheeting weighing 3.5 ounces per square yard and averaging 101 filling yarns and 106 warp yarns to the inch was found most satisfactory.

The cloth was desized by a method developed by the author and Peterson (*29*) in this laboratory. Four 18-inch squares of the desized sheeting were then weighed after conditioning overnight in a room maintained continuously at 70° to 75° F. and 50 per cent relative humidity.[3] Three of the samples were next soiled simultaneously

[3] Through the courtesy of E. O. Reed, the humidity room of the division of tests and technical control, U. S. Government Printing Office, was used for this work.

by agitating for 10 minutes in a small agitator type of washing machine in a suspension of the black constituent. The quantity and medium are indicated in Table 2. In addition, the samples were always treated in the same way with a solution of 30 gm. olive oil, 30 gm. tallow, and 60 gm. mineral oil in 4 liters of carbon tetrachloride. In experiments where Oildag, news ink, lampblack, and benzol soot were used the soiling was done in one application, since carbon tetrachloride was the medium for both the black constituent and the oils. With Aquadag and Cibanone black B, the black was applied in water, and after drying at room temperature, the samples were treated with the solution of oils in carbon tetrachloride.

TABLE 2.—*Brightness of samples soiled with various black substances, compared in a photometer with a milk-glass slide of 88.49 per cent brightness relative to magnesium carbonate (100 per cent)*

Experiment No.	Black constituent of soiling mixture	Quantity (grams)	Medium	Per cent brightness of—										Average per cent increase in brightness		Per cent brightness of unsoiled control		
				Soiled samples					Washed samples									
				Individual samples			Average		Individual samples			Average						
				A	B	C	Each run	Two runs	A	B	C	Each run	Two runs	Each run	Two runs	Original	Washed	Decrease
1	Aquadag (20 per cent graphite).	12.00	Water	25.1	24.8	25.2	25.0	----	39.8	41.5	40.0	40.4	----	15.4	----	80.0	78.3	1.7
2	----do			24.9	25.7	25.0	25.2	25.1	41.1	40.4	39.5	40.3	40.4	15.1	15.2	80.0	78.1	1.9
3	Oildag (12.5 per cent graphite).	1.60	CCl_4	27.2	27.4	26.7	27.1	----	53.6	54.1	51.7	53.1	----	26.0	----	81.5	79.5	2.0
4	----do			27.3	26.1	26.8	26.7	26.9	53.5	52.4	54.1	53.3	53.2	26.6	26.3	82.4	81.0	1.4
5	News ink (10 per cent carbon black).	.50	----do	27.2	26.8	27.5	27.2	----	47.1	46.6	47.3	47.0	----	19.8	----	81.4	80.6	0.8
6	----do			28.7	27.8	27.9	28.1	27.7	49.5	49.2	48.4	49.0	48.0	20.9	20.4	82.2	79.8	2.4
7	Lampblack	.25	----do	24.5	23.0	24.6	24.0	----	42.2	39.4	42.0	41.2	----	17.2	----	79.2	77.8	1.4
8	----do			23.3	22.9	23.1	23.1	23.6	40.9	41.3	42.4	41.5	41.4	18.4	17.8	79.5	77.6	1.9
9	Cibanone black B (10 per cent paste).	10.00	Water	27.6	28.3	27.4	27.8	----	42.3	45.4	40.9	42.9	----	15.1	----	82.1	81.8	0.3
10	----do			29.9	29.1	27.9	29.0	28.4	46.3	44.8	45.0	45.4	44.2	16.4	15.8	82.4	82.2	0.2
11	Benzol soot	.15	CCl_4	30.5	28.7	30.4	29.9	----	47.1	45.2	46.5	46.3	----	16.4	----	82.4	81.0	1.4
12	----do			28.4	27.7	27.9	28.0	29.0	42.7	42.0	42.5	42.4	44.4	14.4	15.4	82.5	81.4	1.1
	Average															81.3	79.9	1.4

The samples were again hung in the humidity room to be weighed the following morning. Brightness values were then read on the soiled samples by means of an optical arrangement including a Martens photometer devised by Bruce (*6*) for the measurement of the hiding power of paints. These values were obtained by placing four thicknesses of the cloth over half of a milk-glass slide, which was used as a secondary standard and had a brightness of 88.49 per cent when calibrated against magnesium carbonate rated as 100 per cent. Four readings were made on each test sample, one approximately in the center of each quadrant. The cloth was so folded that the diagonal of the quadrant was parallel to the ends of the slide.

The washing procedure was the same throughout the experiment. The samples were washed separately in an experimental cylinder

washing machine maintained at constant temperature by a water bath. The cylinder revolved at 50 revolutions per minute, reversing its direction after each revolution. Every sample was washed three times for 10-minute periods in suds consisting of 4 liters of distilled water and 10 gm. of Castile soap, and was then rinsed three times for 10-minute periods in 4 liters of distilled water. A temperature of 75° C. was maintained throughout. There was no wringing during the process. After each bath the samples were removed and hung on the line until the next was prepared. A bank of two washers permitted two samples to be washed at the same time. Then the procedure was repeated with the third sample and the unsoiled control. The samples were dried at room temperature, conditioned overnight in the humidity room, and then weighed and their brightness determined by the photometer.

Table 2 gives the brightness values of the soiled and washed samples. Table 3 gives the percentages of soil on the test samples after soiling and after washing, as determined by weighing.

TABLE 3.—*Percentage of soil on samples soiled with various black substances, determined by weighing under standard conditions of 70° to 75° F. and 50 per cent relative humidity, calculated on the basis of weight of unsoiled cloth*

Experiment No.	Black constituent of soiling mixture	Per cent of soil on—										Average per cent of soil removed		Per cent increase in weight of unsoiled control after washing
		Soiled samples					Washed samples							
		Individual samples			Average		Individual samples			Average		Each run	Two runs	
		A	B	C	Each run	Two runs	A	B	C	Each run	Two runs			
1	Aquadag	5.6	6.2	5.8	5.9	-----	3.2	3.4	3.2	3.3	-----	2.6	-----	0.32
2	----do	5.7	6.0	5.6	5.8	5.8	3.0	3.2	3.0	3.1	3.2	2.7	2.6	.38
3	Oildag	4.6	4.7	5.2	4.8	-----	2.4	2.5	3.0	2.6	-----	2.2	-----	*.17
4	----do	4.8	4.8	4.4	4.7	4.8	3.2	3.6	3.1	3.3	3.0	1.4	1.8	.36
5	News ink	5.2	4.7	4.8	4.9	-----	3.5	3.2	3.4	3.4	-----	1.5	-----	.45
6	----do	4.8	4.9	5.5	5.1	5.0	3.2	3.2	3.5	3.3	3.4	1.8	1.6	.21
7	Lampblack	5.0	5.4	4.7	5.0	-----	3.2	3.4	3.2	3.3	-----	1.7	-----	.51
8	----do	5.4	5.7	5.2	5.4	5.2	3.1	3.2	3.1	3.1	3.2	2.3	2.0	.13
9	Cibanone black B	5.5	4.8	4.9	5.1	-----	3.3	2.8	3.0	3.0	-----	2.1	-----	.15
10	----do	5.1	5.4	5.2	5.2	5.2	3.6	3.8	3.1	3.5	3.3	1.7	1.9	.76
11	Benzol soot	4.3	4.6	4.2	4.4	-----	3.2	3.4	3.3	3.3	-----	1.1	-----	.61
12	----do	4.3	5.2	4.6	4.7	4.5	3.1	3.6	3.3	3.3	3.3	1.4	1.2	.25
	Average	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	.38

* 0.17 per cent represents decrease in weight and soil is not included in the average 0.38.

According to the data in Tables 2 and 3 all the black constituents seem to fulfill the requirements of artificial soiling; that is, the soiling can be reproduced and the effect of washing can be duplicated in successive experiments. A second run in each set checked the first rather well. Samples soiled with Oildag were washed cleaner than any of the others, showing a 26.3 per cent brightness increase compared with a 20.4 per cent increase in the case of news ink and a 15 per cent approximate increase in the case of samples soiled with the other black substances. The unsoiled controls showed practically the same results throughout; that is, an almost insignificant decrease (1.4 per

cent) in brightness and, with the exception of experiment No. 3, a slight increase in weight with washing.

In order that the average brightness of the four quadrants may represent a reliable measure of the brightness of the entire surface of the test sample, good uniformity of depth of soil must exist throughout. The standard deviation was therefore calculated in order to obtain a measure of the variation of the brightness of each quadrant from the arithmetical mean. (Table 4.)

TABLE 4.—*Average brightness value of six samples with two runs for each black constituent, and standard deviations and standard errors of averages*

Black constituent of soiling mixture	Brightness of soiled samples (average of two runs)	Standard deviation	Standard errors [a] $\sigma M=\frac{\sigma}{\sqrt{N}}$ $N=24$	Brightness of washed samples (average of two runs)	Standard deviation	Standard errors $\sigma M=\frac{\sigma}{\sqrt{N}}$ $N=24$
	Per cent			*Per cent*		
Aquadag	25.1	0.63	0.13	40.4	1.34	0.27
Oildag	26.9	.66	.14	53.2	1.09	.22
News ink	27.7	.85	.17	48.0	1.48	.30
Lampblack	23.6	1.02	.21	41.4	1.86	.38
Cibanone black B	28.4	1.21	.25	44.2	2.56	.52
Benzol soot	29.0	1.36	.28	44.4	2.21	.45

[a] σM equals standard error of the mean; σ equals standard deviation; and N equals number of observations.

Obviously the black constituent showing the smallest standard deviation is more highly recommended than the others for use in the artificial soiling mixture. In both soiled and washed samples, the black substances permanently suspended when purchased, exclusive of Cibanone black B, have smaller standard deviations than the dry carbons—lampblack and benzol soot. Of the permanently suspended blacks, Aquadag and Oildag are superior to news ink (carbon black) as regards uniformity within each sample and in respect to their ability to soil three samples uniformly and simultaneously in the same run. Oildag is to be preferred to Aquadag because it can be attached to the fabric together with oils in one application.

Table 4 reveals further that the soiled samples as a group show less variation than the washed samples with every black component. Even soiling is apparently less difficult than even washing with the apparatus used in these experiments.

The reliability of the brightness increase figure, which is an index of washing effect, is of great importance. Since no correlation exists between brightness of spots on soiled and washed samples, respectively, in the close range dealt with, the reliability of this figure can be determined by the formula

$$\sigma d=\sqrt{\frac{\sigma s^2+\sigma w^2}{N_r}}$$

where σd equals standard error of difference, σs equals standard deviation of the soiled samples, σw equals standard deviation of the washed samples, and N_r equals the number of brightness readings made on both soiled and washed samples. N_r equals 24 in all calculations, since four readings are made on each sample and there are six samples in two runs. The value indicating the increase in brightness together with the standard deviation for Oildag is 26.3 ± 0.26, which

means that chances are about two to one that the brightness increase with two runs, three samples each, four brightness readings on each sample, would not vary from the 26.3 by more than 0.26. By contrast with the reliability of the brightness increase figure for Oildag, benzol soot shows 15.4 ± 0.53, and Cibanone black B, 15.8 ± 0.58. Oildag would be selected again because of the greater reliability exhibited by the figure expressing brightness increase.

METHOD OF APPLYING THE SOILING MIXTURE TO THE TEST CLOTH

The selection of a suitable method of applying the soiling mixture involved the study of a number of mechanical devices. It was found that the use of a ball mill, a laboratory mechanical stirrer, and an oscillating shaking machine were not satisfactory on account of the irregular soiling produced. Of these the oscillating shaking machine was most promising.

Better results were produced by placing the soiling mixture in a shallow pan and dipping the sample into it. However, it was found necessary to use a strictly uniform procedure if comparable results were to be obtained. The best method of doing this consisted in immersing the sample for one minute, reversing it, and allowing it to remain immersed for another minute. It was then lifted out and turned once while drying. The distribution of the soil over the surface of the samples was fairly good. The duplicating of the brightness of soil with a second trial was poor with the lampblack constituent but excellent with the Oildag.

Another method, which was unsuccessful but which undoubtedly has possibilities, consists in stretching the fabric over frames which are then fitted into the grooves of a photographic tank. By blowing air into the bottom of this, the soiling mixture can be circulated and kept from settling during the half-hour exposure of the test samples.

The procedure finally selected for all the soiling done in this laboratory involved the use of a small agitator washing machine. In the bottom of the tub is a four-bladed aluminum agitator which is operated by a hand lever. The revolving device is reversed after each revolution and thus carries the cloth first in one direction and then in the other. By use of this machine it has been possible to soil each test sample uniformly, to soil three pieces similarly at the same time, and to duplicate a soiling of three samples with the same treatment.

QUANTITATIVE DETERMINATION OF WASHING EFFECT

Bergell (*4*), Lindner (*23*, *24*), Parker (*28*), and Schiewe and Stiepel (*30*) have suggested that the amount of soil removed from samples in experimental laundering studies may be determined by weighing, although Heermann (*14*) specifically advises against this method. In order to test the feasibility of this suggestion the two standard methods of weighing, in a constant humidity room and in a conditioning oven, were compared. Photometric readings and weighings in a constant humidity room had been made on all samples up to this point.

TABLE 5.—*Brightness of Oildag-soiled samples, unheated and heated, compared in a photometer with a milk-glass slide of 88.49 per cent brightness relative to magnesium carbonate (100 per cent)*

Experiment No.	Black constituent of soiling mixture	Per cent brightness of—										Average per cent increase in brightness		Per cent brightness of unsoiled control		
		Soiled samples					Washed samples									
		Individual samples			Average		Individual samples			Average						
		A	B	C	Each run	Two runs	A	B	C	Each run	Two runs	Each run	Two runs	Original	Washed	Difference
3	Oildag, unheated	27.2	27.4	26.7	27.1	----	53.6	54.1	51.7	53.1	----	26.0	----	81.5	79.5	−2.0
4	....do	27.3	26.1	26.8	26.7	26.9	53.5	52.4	54.1	53.3	53.2	26.6	26.3	82.4	81.0	−1.4
13	Oildag, heated	30.3	29.6	29.4	29.8	----	34.2	34.1	34.9	34.4	----	4.6	----	82.1	81.3	−0.8
14	....do	30.8	30.6	29.7	30.4	30.1	36.0	35.5	34.8	35.4	34.9	5.0	4.8	81.4	81.5	−0.1

The samples in the experiment with heated Oildag (Table 5, experiments 13 and 14) were subjected to the same soiling and washing procedures as those with unheated Oildag (Table 5, experiments 3 and 4). The only difference was that, in the former, weighings were made in each case with the samples in a bone-dry condition. This was accomplished by heating in a conditioning oven at 105° to 110° C. for four hours with a fan running. Brightness values of the samples are recorded in Table 5 and the weighings in Table 6.

TABLE 6.—*Percentage of soil on Oildag-soiled samples, unheated and heated, determined by weighing in a room maintained at 70° to 75° F. and 50 per cent relative humidity, and in a conditioning oven at 105° to 110° C., respectively, calculated on the basis of weight of unsoiled cloth*

Experiment No.	Black constituent of soiling mixture	Per cent soil on—										Average per cent of soil removed		Per cent difference in weight of unsoiled control after washing
		Soiled samples					Washed samples							
		Individual samples			Average		Individual samples			Average				
		A	B	C	Each run	Two runs	A	B	C	Each run	Two runs	Each run	Two runs	
3	Oildag, unheated	4.6	4.7	5.2	4.8	----	2.4	2.5	3.0	2.6	----	2.2	----	−0.17
4	....do	4.8	4.8	4.4	4.7	4.8	3.2	3.6	3.1	3.3	3.0	1.4	1.8	+0.36
13	Oildag, heated	4.5	4.4	4.8	4.6	----	2.8	2.8	2.8	2.8	----	1.8	----	−0.12
14	....do	4.5	4.6	5.0	4.7	4.6	2.7	2.9	3.1	2.9	2.8	1.8	1.8	−0.04

A comparison of the average percentage brightness increase of the three samples in each set shows a 26 per cent approximate increase in the unheated against a 5 per cent increase in the heated samples. Mere visual evaluation with the naked eye agrees with this finding, and yet weighings show 1.8 per cent of soil removed in each case. Here, as also in the humidity room weighings, very poor correlation exists between brightness values and percentage soil on the weight of the cloth. Descending brightness values should give ascending per-

centages representing soil on the weight of the cloth. The two standard methods of weighing textile fabrics fail to be useful in determining the results of laundering experiments. The humidity room permits weighing under standard conditions but requires too long a time for the samples to reach equilibrium. Jarrell (*22*) finds that 48 hours are required for the attainment of equilibrium when paper is transferred from 35 per cent to 65 per cent relative humidity and about 6 days when changed from 65 per cent to 35 per cent. In contrast to this the conditioning oven furnishes speed but is also unreliable as a method of determining true experimental results. In addition, the soil is apparently fastened to the fabric so that washing effects can not be studied.

A photometric method was the only other used in this laboratory for determining the amount of soil removed. Bruce's (*6*) arrangement of Martens photometer is reasonably satisfactory for this purpose in that it permits any desired number of readings over the surface of the cloth. The great disadvantage lies in the small area that each brightness reading represents. This is only one-half of a circle 3 cm. in diameter. In the writer's experiments the soil has been evenly enough distributed to allow an average of four readings, one in each quadrant, to represent the reflection value of the entire surface of the 18-inch square. However, an instrument may be arranged to read a larger surface of the test sample. Brightness readings are preferred to weighings as a method of determining the results of the washing process.

SUMMARY

A comparative study of the black constituents suggested for use in experimental soiling methods results in the recommendation of Oildag for this purpose. Samples soiled with mixtures containing this material are of uniform and reproducible brightness.

The cotton sheeting used in these experiments was of suitable yarn composition and weave to permit a uniform distribution of the dirt mixture after soiling and after wash ng.

The soiling mixture now being used by this bureau in experimental laundering studies contains the following constituents: Oildag, olive oil, tallow, and mineral oil. Representatives of all constituents of natural soiling are present with the exception of albuminous matter and stains. Albuminous matter, if included, would limit the temperature range of experimental work.

A small agitator type of washing machine is used in applying the mixture to the fabric. It deposits the soil uniformly over the surface of each sample, furnishes samples of approximately the same brightness, and with the same procedure enables the production of a second set of the same uniformity and brightness.

Weighing, either in a humidity room or in a conditioning oven, was found to be an unsatisfactory method of determining the amount of soil removed. A photometric method is therefore now being employed.

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THE CHEMICAL COMPOSITION OF GIRASOLE AND CHICORY GROWN IN MINNESOTA[1]

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INTRODUCTION

In connection with the utilization of the girasole, or Jerusalem-artichoke, *Helianthus tuberosus*,[4] and chicory, *Cichorium intybus*, as truck crops and in possible sugar manufacture, the chemical composition is of interest. In the case of the girasole a knowledge of the composition of the tops is important because of the value of this portion of the plant as a food for stock. In the present paper are reported analyses of the crops grown at University Farm, St. Paul, Minn., in 1927.

The material was analyzed according to the Official Methods (*1*) [5] except the sugars in girasole tubers and chicory roots. These were determined by Ost's cupro-carbonate method as modified by Nyns[6] and reviewed by Oliver,[7] and Traub, Thor, Willaman, and Oliver.[8]

GIRASOLE

Analyses of the various constituents of girasole grown under European conditions have been reported by König (*8, v. 1, p. 729; v. 2, p. 900–901*), Fingerling-Kellner (*7*), and by Müntz and Girard as quoted by Garola (*4*). The present analyses show the composition of girasole under Minnesota conditions. For these analyses four varieties were chosen—Portland, Mammoth French white, a purple variety, and a variety sent out by the United States Department of Agriculture, here referred to as U. S. D. A. The plants were grown on rich sandy loam. A comparison of the composition of tubers of the four varieties are presented in Table 1.

In these tubers there is, generally speaking, a rise in the dry matter, carbohydrate fractions, and total nitrogen from August 30 to November 3. On the latter date the highest total sugar content was a little more than 15 per cent in the Portland and Mammoth French white varieties. The sugar content of the U. S. D. A. and purple varieties was slightly lower.

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[3] Thanks are due S. C. Nelson, E. S. Miller, and C. E. Steinbauer for competent assistance in certain of the chemical determinations.

[4] *Helianthus tuberosus* is not an artichoke and is not native to Palestine; therefore "Jerusalem-artichoke" is a misnomer, and is being replaced by the simpler term "girasole." See L. H. Bailey (*2, p. 446–449*).

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The important consideration in the manufacture of fructose is the ratio of fructose to total sugars (*6*). In the ratio of fructose to other sugars present Mammoth French white ranks highest, followed by U. S. D. A., Portland, and the purple variety, in the order named.

To determine the relative value of the various parts of the whole plant, analyses were made of the tubers, leaves, and stems of one variety. The data covering the composition of tubers, leaves, stems, and entire tops of the Portland variety are summarized in Table 2.

TABLE 1.—*Composition of the tubers of four girasole varieties grown on sandy loam at University Farm, St. Paul, Minn., and analyzed August 30 and November 3, 1927*

Variety	Date analyzed	Composition expressed as percentage of green weight							
		Moisture	Dry matter	Fructose	Glucose	Total sugar	Ash	Crude protein	Crude lipides[a]
Portland	Aug. 30	84.6	15.3	6.72	3.61	10.33	1.09	1.55	0.26
	Nov. 3	79.8	20.1	9.99	5.06	15.05	1.08	2.56	.....
Mammoth French white	Aug. 30	83.5	16.4	7.52	3.43	10.95	1.24	1.75	.24
	Nov. 3	79.1	20.8	10.3	4.79	15.09	1.13	2.43	.17
U. S. D. A	Aug. 30	84.6	15.3	7.29	3.72	11.01	1.00	1.50	.....
	Nov. 3	80.2	19.7	9.33	4.43	13.96	1.16	2.62	.....
Purple	do	78.9	21.0	9.38	4.74	14.12	1.18	3.06	.....

Variety	Date analyzed	Composition expressed as percentage of dry weight						Ratio of—	
		Fructose	Glucose	Total sugar	Ash	Crude protein	Crude lipides	Fructose to glucose	Fructose to total sugar
Portland	Aug. 30	43.6	23.4	67.1	7.0	10.0	1.6	1.86	0.65
	Nov. 3	49.5	25.0	74.6	5.3	12.7	.....	1.97	.66
Mammoth French white	Aug. 30	45.8	20.9	66.7	7.5	10.5	1.4	2.19	.69
	Nov. 3	49.4	22.9	72.3	5.4	11.6	.8	2.15	.68
U. S. D. A	Aug. 30	47.4	24.2	71.6	6.5	9.7	.....	1.96	.66
	Nov. 3	48.3	22.4	70.7	5.8	13.3	.....	2.15	.68
Purple	do	44.6	22.5	67.1	5.6	15.6	.....	1.98	.66

[a] Cloroform extract.

TABLE 2.—*Chemical composition of tubers, leaves, and stems of girasole plants of the Portland variety, grown at University Farm, St. Paul, Minn., in 1927*

Determination	Percentage of green weight in—				Percentage of dry weight in—			
	Tubers[a]	Leaves[b]	Stems[b]	Entire top[b]	Tubers[a]	Leaves[b]	Stems[b]	Entire top[b]
Moisture	79.8	75.8	66.1	69.9	.....	.....	.....	.....
Dry matter	20.1	24.1	33.8	30.0	.....	.....	.....	.....
Reducing sugars	.....	1.72	1.96	1.86	.....	7.14	5.79	6.22
Nonreducing sugars	.....	2.03	6.03	4.44	.....	8.37	17.81	14.79
Total sugars	[c] 15.04	3.75	7.99	6.30	74.61	15.51	23.60	21.00
Starch	.....	1.02	6.25	4.18	.....	4.21	18.47	13.91
Pentosans	.83	2.07	.42	1.07	3.90	8.56	1.25	3.59
Protein (×6.25)	2.56	3.75	.93	2.00	12.75	15.43	2.81	6.87
Ash	1.08	3.58	.74	1.87	5.37	14.80	2.18	6.21
Crude lipides (chloroform extract)	.....	.17	.04	.....	.....	.07	.12	.....

[a] Nov. 3. [b] Oct. 11. [c] Total water-soluble carbohydrates.

The dry matter in the leaves, stems, and entire tops of the girasole is relatively higher than that in the tuber. The tubers contain relatively much more sugar than the leaves, stems, and entire top. The starch content of the leaves, determined by the diastase method, is relatively lower than that of the stem portion. The pentosan, nitrogen, and ash content of the leaves is relatively much greater than that of the stem. In the case of the tubers, the pentosan value was determined on the residue of a sample after thorough extraction with 80 per cent alcohol and hot water. The amount recovered is the same as that reported by Müntz and Girard (*4*).

The total sugar content of the entire top is a little more than 6 per cent of the green weight and 21 per cent of the dry weight. The starch content is more than 4 per cent of the green weight and more than 13 per cent of the dry weight. The protein content is 2 per cent of the green material and more than 6 per cent of the dry material. In the utilization of the tops for feeding and other purposes these facts are important.

A comparison of the Minnesota analyses with those of Müntz and Girard (*4*) in Europe has been attempted in Table 3. The table shows that girasole tubers and tops grown under Minnesota conditions compare favorably with those grown in Europe.

TABLE 3.—*Chemical composition of mature girasole tubers, tops, and leaves, as determined by the writers and by Müntz and Girard (4)*

[Values are expressed in percentage of green weight]

Determination	Composition of tubers as shown by—		Composition of top as shown by—		Composition of leaves as shown by—	
	Present data	Müntz and Girard	Present data	Müntz and Girard	Present data	Müntz and Girard
Moisture	79.8	81.9	69.9	83.0	75.8	84.2
Dry matter	20.1	18.1	30.0	17.0	24.1	15.8
Reducing sugars	(a)		1.86		1.72	
Nonreducing sugars			4.44		2.03	
Total sugars	[b] 15.04	[c] 11.05	6.50		3.75	
Starch			4.18		1.02	
Pentosans	.83	.83	1.07	1.99	2.07	1.39
Protein	2.56	1.46	2.00	2.54	3.75	3.08
Ash	1.03	1.50	1.87	2.89	3.58	3.31

[a] Blanks indicate that no figures were reported.
[b] Total water-soluble carbohydrates.
[c] Inulin plus sugar.

Typical acre yields of girasole tubers of the Portland variety for the season 1927 in Minnesota, based upon 1/16-acre plots on rich sandy loam and silt loam, are, for sandy loam, 11.50 tons per acre, and for silt loam 5.25 tons per acre. The crop on the sandy loam was planted the middle of April, and that on silt loam the first week in May, and this difference in date of planting may account in part for the great variation in yield on the two soils. These yields compare favorably with those reported for France by Shoemaker (*9*). On October 1 the plants on silt loam averaged 7½ feet in height and were just, beginning to flower; on sandy loam on the same date the plants averaged 8 feet and were in full bloom.

CHICORY

Analyses of chicory have been reported by Tatlock and Thomas (*11*) and others who have studied chicory as a substitute for coffee. Colin (*3*), Sirot and Joret (*10*), and Grafe and Vouk (*5*) have reported on inulin in chicory. These analyses are not strictly comparable with those reported in this paper, where chicory is considered as a source of fructose.

TABLE 4.—*Comparison of the chemical composition of the roots of two varieties of chicory grown at University Farm, St. Paul, Minn., and analyzed September 15 and October 14, 1927*

Variety	Date analyzed	Composition in percentage of green weight						
		Moisture	Dry matter	Fructose	Glucose	Total sugar	Ash	Crude protein
Large-rooted Magdeburg	Sept. 15	81.7	18.2	10.13	3.53	13.66	0.72	1.25
	Oct. 14	79.4	20.5	10.58	5.99	16.57	.73	1.37
Westland's strain	Sept. 15	80.6	19.3	10.68	3.47	14.15	.79	1.20
	Oct. 14	77.6	22.3	10.79	6.63	17.42	.71	1.62

Variety	Date analyzed	Composition in percentage of dry weight					Ratio of *	
		Fructose	Glucose	Total sugar	Ash	Crude protein	Fructose to glucose	Fructose to total sugar
Large-rooted Magdeburg	Sept. 15	55.47	19.33	74.80	3.96	6.8	2.87	0.74
	Oct. 14	51.51	29.16	80.67	3.57	6.8	1.76	.64
Westland's strain	Sept. 15	55.25	17.95	73.20	4.08	6.1	3.08	.75
	Oct. 14	48.23	29.64	77.87	3.19	7.3	1.63	.62

* On a green-weight basis.

A comparison of two superior varieties of chicory, Large-rooted Magdeburg and Westland's strain, grown on silt loam at University Farm, St. Paul, Minn., is presented in Table 4. This table shows that the increase in dry matter between September 15 and October 14 amounts to 2.3 and 3.0 per cent of the green weight for Large-rooted Magdeburg and Westland's strain, respectively. The table shows also that there is a perceptible increase in total sugars in both varieties from September 15 to October 14, amounting to about 3 per cent of the green weight. This difference is due to a relatively greater increase of sugar other than fructose, since the latter value remains practically constant. The absolute amount of water-soluble carbohydrates in both varieties at maturity, October 14, is practically the same, 16.57 and 17.42 per cent, of the green weight. The fructose values for these two varieties are 10.58 and 10.79 per cent of the green weight. The ash content for both varieties is about 0.7 and the protein content about 1.2 per cent.

In connection with the ratios of fructose to glucose and fructose to total sugars, on a green-weight basis, it should be noted that the highest ratios are reached at the earlier date, September 15. By October 14, because of the relative increase in glucose, the ratios decrease markedly.

COMPARISON OF GIRASOLE AND CHICORY

A comparison of the fructose and total sugar content of chicory on September 15 and girasole at its best on November 3 shows that, under Minnesota conditions, chicory has consistently a higher fructose and total sugar content. The ratios of frustose to glucose and fructose to total sugars are also higher for chicory. (Tables 1 and 4.) These considerations are important in the possible utilization of chicory as a source of fructose.

SUMMARY

Two of the best varieties of the girasole grown, Portland and Mammoth French white, contain a little more than 15 per cent sugar on a green-weight basis.

Mammoth French white has the higher ratio of fructose to total sugars.

The sugar content of the tubers is relatively greater than that of the tops.

The total sugar and starch content of the tops is more than 10 per cent of the green and more than 30 per cent of the dry weight.

As a source of fructose, chicory is apparently as desirable as, or more desirable than, girasole.

Chicory apparently reaches its maximum fructose content relatively earlier (September in Minnesota) than girasole (November in Minnesota).

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